EFFECTS OF PARENTAL AND INCUBATION ENVIRONMENTS ON MERISTIC VARIATION IN *RIVULUS MARMORATUS*

BY

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ABSTRACT

Adults from one clone of *Rivulus marmoratus* were held at 25C or 30C, and their offspring were either incubated at the parental holding temperature until after hatching or transferred to the alternate temperature after various periods of development. Effects of parental temperature transfers and of transfers of developing embryos between temperatures indicated that for a given incubation temperature a parental temperature of 25C produces significantly more vertebrae and caudal and pectoral rays in offspring than one of 30C. Numbers of dorsal and anal rays are not significantly affected by parental temperature.

Temperature breaks showed that all meristic series examined are thermolabile shortly after fertilization. Vertebral counts are determined within 80 h at 25C, caudal ray counts within 72 h, and pectoral, dorsal and anal rays probably within 144-192 h. Patterns of response to reciprocal break experiments were roughly mirror images. Early temperature breaks produced an overcompensation in vertebral and pectoral and caudal ray counts. Temperature breaks produced a paradoxical reaction in dorsal ray counts if applied early, or an overcompensation if applied late. Temperature breaks applied at most times during the thermolabile period produced a paradoxical reaction in anal ray counts.

Oviposition during stages 18 (7-8 somites) to 21 (first body movements) was associated with a high incidence of vertebral fusions.

Anal ray counts increased and vertebral and possibly pectoral ray counts decreased with increasing duration of intraparental incubation. All meristic counts were increased significantly by transfers from brackish to freshwater at some developmental stages. Transfers from light to darkness early in development significantly decreased vertebral counts, those late in development significantly decreased pectoral, anal and caudal ray counts. Patterns of response to light breaks indicated extralimitary responses in pectoral, anal and caudal ray counts and possibly in vertebral counts. Offspring of parents which had just begun to lay eggs had significantly fewer meristic parts (except caudal rays) than did those of "experienced" parents. Fish with grandparents held at 30C may have more vertebrae than those with grandparents held at 25C.

Possible mechanisms involved in the influences of parental reproductive history, grandparent temperature history, and incubation and parental environments on meristic counts are discussed.

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1. INTRODUCTION

Environmental conditions experienced during development are known to influence the final numbers of meristic parts (such as vertebrae and fin rays) in fish (see the reviews of Barlow (1961), Fowler (1970) and Garside (1970)). In most studies of meristic variation, however, meristic characters are tacitly assumed to be unaffected by the environment experienced by parents before the conception of their offspring. Evidence that early embryonic development may be controlled by cytoplasmic substances produced during oogenesis (e.g., Davidson 1968) casts suspicion on the validity of this assumption. Indeed, Dentry (1976) reported strong indications of an influence of parental temperature history on meristic counts in offspring in zebrafish (also see Dentry and Lindsey 1978).

The interpretation of Dentry's results is unfortunately hampered by heritable variation in meristic responses to incubation and parental temperatures. The present study investigates the influence of parental temperature on meristic counts using fish belonging to a single clone of the self-fertilizing cyprinodont species *Rivulus marmoratus*. Due to the genetic uniformity of this material, the heritable variation which plagued Dentry's experiments is avoided in this study. Possible influences of parental reproductive history and grandparent temperature experience on meristic counts were also

examined when it became apparent during the course of the study that these factors might be additional "prefertilization" sources of meristic variation.

A second purpose of this study was to investigate some effects of the incubation environment on meristic counts. Generalizations which predict or explain meristic responses to incubation environments have been elusive despite extensive study. For example, patterns of meristic response to varied sustained levels of incubation temperature (the most extensively studied environmental factor) may be declivous, aclivous, V-shaped or chevron-shaped (Tåning 1952, Lindsey 1954, Ali and Lindsey 1974, and others). Responses differ among different meristic series in a given species and among species for a given meristic series. A meristic response model recently developed by Arnason, Choy and Lindsey (1978) casts illuminating light on these apparently contradictory results. This model suggests that environmental factors such as incubation temperature influence the numbers of meristic parts through differential effects on rates of growth and differentiation[1], an idea first implied by Hubbs (1926). The model is able to simulate the diverse types of meristic responses noted above (C.C. Lindsey, pers. comm.), offering a simple explanation for the mechanisms underlying the superficially complex effects of incubation environments on meristic counts.

An impressive feature of the meristic response model of Lindsey and Arnason is its ability to describe responses to temperature

[1]The term "differentiation" is used in this study in the general sense of a progressive specialization of cells.

breaks and pulses during development. (Temperature breaks are defined as permanent transfers of embryos from one temperature to a second temperature at which they are kept until the completion of development; temperature pulses refer to temporary transfers of embryos from one temperature to a second temperature, followed by return to the original temperature for completion of development.) Temperature breaks and pulses sometimes produce "extralimitary" meristic counts (mean counts outside of those produced by either sustained temperature). Extralimitary responses are often ascribed to metabolic upsets or shocks due to transfer between temperatures (e.g., Hallam 1974) but are also predicted by the meristic response model (Arnason *et al.* 1978).

The meristic response model also implies prolonged periods of thermolability, beginning early in development. Extended thermolabile periods beginning shortly after fertilization are suggested for vertebral counts by the few sufficiently intensive temperature break experiments reported to date (Ali and Lindsey 1974, Hallam 1974). However, in these experiments, fin ray responses were either unexamined (Hallam 1974) or perplexing (Ali and Lindsey 1974).

The present study reports vertebral and fin ray responses to temperature, salinity and light breaks in *R. marmoratus*. The salinity and light break experiments are not extensive but provide preliminary information regarding the influences of these little studied environmental factors on meristic counts. The more extensive temperature

break experiments include a close spacing of breaks at early and intermediate developmental times, avoiding the confusion generated by insufficient transfer times in most other studies. Both vertebral and fin ray counts showed clear patterns of response to temperature breaks interpretable in terms of the meristic response model of Lindsey and Arnason. The temperature break experiments are also integrated with experiments involving different holding temperatures of parents, and lead to the conclusion that the parental environment before fertilization does have a significant effect on meristic characters of offspring.

2. MATERIALS AND METHODS

2.1 Experimental Fish

Rivulus marmoratus Poey is a small cyprinodont fish native to Florida and the Caribbean (Lindsey and Harrington 1972). It is unique among fishes in normally reproducing uniparentally via synchronous hermaphroditism with internal self-fertilization (Harrington 1961, 1963). Apparently striking phenotypic plasticity (Lindsey and Harrington 1972, Harrington and Crossman 1976a) and genetic uniformity commended it as a promising species for investigation of environmental influences on meristic variation.

Low individual egg production necessitated the use of almost 100 parents in this study. All fish were uniparental descendants of one hermaphrodite (DS) caught by R.W. Harrington in Vero Beach, Florida in 1960. The first group of parents, 35 fish of the 28th and 29th laboratory-reared generations of the DS clone, were shipped as eggs by air mail from Vero Beach to Winnipeg in June 1976. The F_1 and F_2 offspring of these fish contributed the remainder of the parents used.

Uniparental reproduction by self-fertilization for 28 or more generations of laboratory culture assures virtual to complete isogenicity and homozygosity in the fish used in this study. In addition, it is likely that many generations of selfing in the wild in Florida have contributed to the genetic uniformity of the stock used (see

Lindsey and Harrington 1972). The expectation of genetic uniformity is confirmed by immunological evidence. Tissue transplantation tests have demonstrated that the fish of a clone are isogenic (Kallman and Harrington 1964) and homozygous (Harrington and Kallman 1968), at least with regard to their histocompatibility genes. This high degree of genetic uniformity eliminates genetic variability and selective mortality among different genotypes as possible major sources of meristic variation observed in this study.

The benefits of genetic uniformity and meristic plasticity are partially offset by several disadvantages attending the mode of reproduction in *R. marmoratus*. The chief disadvantage is egg retention within parents for from several minutes to about 4 days following fertilization. Harrington (1963) reported a mean duration of intraparental incubation of 10 h at 25C. Variable egg retention within parents severely restricted experimental design and introduced several possible extraneous sources of meristic variation.

Individual egg production is low and very variable. For example, Harrington (1971) noted that average egg production of 21 DS clone hermaphrodites varied from 9 to 34 eggs per 28 day period. Individuals often exhibit long periods without any egg production. About 75-80% of the eggs laid may be eaten by the parent unless immediately retrieved by pipette (Harrington 1971). The proportion of uneaten eggs which are unfertilized or aborted (presumably due to delayed fertilization (Harrington 1971)) may be high (Harrington (1971) reported values of 10-80%). Low percentages of fertile and viable

eggs may reflect inadequate testicular activity (Harrington 1971).

Although oviposition is most frequent between 0900 and 1200 depending upon photoperiod, it may occur any time between sunrise and sunset under natural photoperiods (Harrington 1963). Efficient egg collection is hampered by this absence of marked periodicity in oviposition. Given an appropriate photoperiod and water temperature, spawning may occur throughout the year in laboratory cultures. Egg production is greatest at about 14 h light per day (Harrington 1968). Unfortunately, presumed testicular activity (indicated by the proportion of eggs that are fertile and viable) is greatest at shorter daylengths, presumably due to hormonal inhibition by high ovarian activity at the longer daylengths (Harrington 1968).

2.2 Maintenance of Adults

All adult fish were held in about 40% sea water[1] (15.1‰ salinity) containing 1 drop/l of 1% methylene blue solution. Each parent was kept in isolation, either in about 600 ml of standing water in a covered 950 ml glass jar or in about 2-3 l of standing water in a covered 1 gal glass aquarium (22 x 14.5 x 15 cm). Some breeding fish were contained in nylon mesh breeding baskets (16.5 x 12.5 x 13.5 cm or 9.5 x 9.5 x 13.5 cm) suspended inside aquaria to reduce egg consumption. However, considerable egg consumption persisted despite the use of breeding baskets, probably because ovipositions most commonly occur near the surface (Harrington 1963). Water levels were

[1]Synthetic sea water was made by adding "Instant Ocean" salt (Aquarium Systems, 33208 Lakeland Blvd., Eastlake, Ohio) to dechlorinated tap water.

reduced in some aquaria (reducing water volume to 1-2 l) in an attempt to reduce egg consumption.

Fish were fed *Artemia* nauplii *ad libitum* once daily, usually 6 days per week. Debris was siphoned from the bottom of jars and aquaria as necessary, usually once or twice per week. Water removed during cleaning was replaced with fresh 40% sea water. Water was changed completely every few weeks.

Adults were held at about 25C or 30C, in one of four locations. Water temperature was maintained at about the above levels by regulating room temperature. Water temperatures were recorded at least once daily, 5 or 6 days per week. The range of water temperature and frequency of temperatures outside 25.0 ± 0.5C or 30.0 ± 0.5C are shown for each room in Table 1. Most adults were kept at about 25C in a "Coldstream" controlled environment room (rm. 407) equipped with its own thermostatically-controlled heating and chilling systems. The majority of eggs used in this study were collected from parents in this room between 5 January and 13 December, 1977. Some adults were kept less precisely at about 25C in a separate laboratory, room 413, to ensure the survival of the colony should room 407 malfunction. Some eggs collected from these fish between 9 October 1976 and 11 March 1977 and in February 1978 were used in this study. Other adults were maintained at about 30C, either in a large incubation chamber (designated rm.406) or in a second Coldstream controlled environment room (rm. 410). Five fish were moved from 25C to the first chamber on 1 September, 1976.

TABLE 1. Ranges and frequencies of deviations greater than 0.5C from nominal temperatures for water temperatures in parent holding rooms during and shortly before periods of egg collection.

Room	Nominal water temperature	Period	Range (C)	>+ 1.0	+(0.6-1.0)	-(0.6-1.0)	-(1.1-1.5)	-(1.6-2.0)	-(2.1-2.5)
407	25C	1 Jan-13 Dec.1977	23.6*-26.1	2	30	29	5	0	0
413	25C	9 Sept. 76-11 Mar. 77	23.1 -25.5	0	0	68	26	7	0
		Jan., Feb., 1978	24.6 -25.8	0	3	0	0	0	0
406	30C	1 Sept. 76-20 Apr. 77	27.5 -31.5	2	8	25	16	2	3
410	30C	19 Sept. -2 Dec. 1977	28.6 -30.7	0	2	3	2	0	0

*Water temperature fell to 23.0C in rm. 407 on May 7 due to accidental manipulation of the thermostat. No eggs were collected on this day or in the following week.

Eggs laid by these fish between 17 October 1976 and 20 April 1977 were used in this study. Other fish were transferred from 25C in room 407 to 30C in room 410 on 19 September 1977. Eggs were collected from these fish between 20 September and 2 December, 1977.

All fish experienced 14 h of artifical illumination per day. Intensities of illumination were about 10 - 50 ft.-c. in room 407, 15 - 100 ft.-c. in room 410, and 25 - 85 ft.-c. in chamber 406. In room 413, intensities were about 40 - 50 ft.c. until May 1977, when they were increased to 80 - 85 ft.c.

2.3 Collection and Care of Eggs and Fry

Breeding fish were checked for eggs at least once daily (usually between 0900 and 1000), 5 or 6 days per week. During some periods checks were made several times each day. "Nonbreeding" fish were usually checked at least weekly, and often daily, for the onset of egg laying.

Eggs were incubated in either freshwater or 40% sea water, both containing 1 drop/l of 1% methylene blue solution. Extra-parental incubation was in freshwater in the main experiments of this study (i.e., the parental temperature and temperature break experiments). Variable duration of extraparental incubation in freshwater introduced an additional possible source of meristic variation. Despite this disadvantage, freshwater incubation was initially used so that results could be compared to and possibly augmented by those of previous

studies of meristic variation in *R. marmoratus* (Lindsey and Harrington (1972) and Harrington and Crossman (1976a) both maintained parents in 40% sea water but incubated eggs in freshwater). Minimal mortality conferred a second advantage to freshwater incubation (E.S. Harrington, pers. comm.). Some workers (e.g., Fahy and O'Hara 1977) considered incubation salinity a relatively insignificant source of meristic variation in fishes. A series of eggs was reared in 40% sea water to ascertain whether this was the case in *R. marmoratus*.

Eggs used only to maintain breeding stock were incubated in freshwater in covered Petri dishes (14 x 90 mm or 22 x 95 mm) at about 25C in room 407 or, rarely, room 413. Eggs used for meristic counts were incubated in containers immersed in 175 l controlled-temperature tanks. Water temperatures in the tanks were maintained usually with ± 0.01C of either 25.0C or 30.0C. On rare occasions, temperatures deviated as much as ± 0.02C from the nominal temperatures. Eggs experiencing fluctuations greater than 0.03C due to equipment or power failure were discarded. Incubation tanks received a constant trickle of water a few degrees below the desired temperature. Final water temperatures were maintained by 1000 watt heaters, "YSI" thermisters and custom-made electronic temperature controllers. Thermal stratification within tanks was prevented by vigorous aeration.

Before May 1977, eggs placed in the controlled-temperature tanks were grouped by embryonic stage and incubated in lots of one to about twenty in about 400 ml of standing water in 950 ml glass jars

covered by Petri dish lids. After mid-May 1977, to retain more of the history known for each egg, eggs were reared individually or rarely in groups of less than five in about 25 ml of standing water in 8 - 10 dram glass vials (22 x 95 mm or 29 x 81 mm). Up to 8 vials were immersed in water in each jar in the water baths.

All eggs experienced 14 h of artifical illumination daily during extraparental incubation. Light intensity 10 cm above the water surface of the incubation baths varied between 32 and 80 ft.c. in the period 1 October 1976 to 1 May 1977 and between 45 and 115 ft.c. thereafter. Intensities incident on the eggs were considerably lower.

The embryonic stages of fish used for meristic counts were identified upon collection using a binocular microscope and the schedule of developmental stages described by Harrington (1963, 1968) and based on the similarly numbered stages described for *Fundulus heteroclitus* by Oppenheimer (1937). Initially, all eggs from parents at 25C were examined at 25.0 ± 0.5C in freshwater in a Petri dish. Temperatures rarely fluctuated outside the above range during the brief time required for stage identification. Before the summer of 1977, eggs collected from parents at 30C were examined in a Petri dish in freshwater initially at 30.0C. During the 10-15 min or less required for handling and stage identification, water temperature in the dish dropped gradually, often to a minimum

of 25 - 26C.[1] Eggs collected from parents at 30C after April 1977 were examined in a Petri dish immersed in a circulating water bath fitted over the microscope stage in order to maintain water temperature at 30.0C. This bath was also often used for stage identifications at 25C during the summer of 1977; all egg examinations after the summer of 1977 were performed using the bath.

Nominal developmental stages were converted to approximate times since fertilization using the schedule published by Harrington (1963) for stages 1 - 24, unpublished data supplied by R.W. Harrington for stages 25 - 30, and timings recorded by A. Brett for later stages. Hours to midstage were used for most stages and groups of successive stages. Averages of the estimated developmental times of individual eggs were used for lots containing eggs of more than one nominal stage. Approximate developmental times were obtained for eggs at 30C by multiplying corresponding developmental times at 25C by 0.817. This factor, undoubtably only a rough approximation, was derived from timings recorded by D. Elliot and A. Brett of eggs developing at 30C (after various durations of intraparental and extraparental incubation at 25C). Table 2 lists the developmental time assigned to each nominal stage at 25C and the definitive characteristics of each stage.

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[1]Preliminary experiments suggested that brief temperature pulses of 10 - 30 min duration have no effect on vertebral or fin ray numbers in *R. marmoratus*. Taning (1952) found no effect of somewhat longer (2 day-degrees) pulses on meristic counts in trout. The meristic response model of Arnason *et al.* (1978) also predicts little or no effect of such brief temperature pulses.

TABLE 2. Developmental stages of *Rivulus marmoratus* (modified from Harrington 1963).

Nominal Stage	Developmental time (h:min.) assigned at 25C	Description
1	1:00	Just fertilized; chorion separating from vitelline membrane; yolk vesicles disappearing; polar cap not undercut.
2	2:30	1 cell; undercut polar cap
3	3:30	2 cell
4	4:00	4 cell
5	4:45	8 cell
6	5:30	16 cell
7	6:15	32 cell; produced by first latitudinal cleavage
8a	7:30	64 cell
8b	8:30	128 cell
8c	9:00	256 cell
8d	9:30	Early high blastula
9	10:15	Late high blastula
10	10:45	Flat blastula
11	12:00	Expanding blastula
12a	15:45	Germ ring and embryonic shield first visible
12b	18:30	Blastoderm 1/3 over yolk surface

Table 2 continued...

Table 2 Continued...

Nominal Stage	Developmental time (h:min.) assigned at 25C	Description
13a	21:30	Blastoderm ½ over yolk surface
13b	23:00	Blastoderm 2/3 over yolk surface
13c	24:00	Blastoderm 3/4 over yolk surface
13d	25:15	Embryonic shield condenses to form the keel; the latter is about 1/8 to 1/6th the circumference of the egg in length
14	28:15	Keel clearer, almost ¼ the circumference of the egg in length.
15	33:30	Blastopore closes; keel ¼ or more the circumference of the egg in length; rudimentary cephalic development apparent.
16	36:45	Optic vesicles first visible as expansions of the forebrain
17	39:45	Optocoeles develop; 3-4 pairs of somites present
18	46:00	Optocoeles connect across brain; optic cup discernible; auditory placodes form; about 7-8 pairs of somites present
19-20a	52:45	Lens develops
20b	56:45	Heart pulses
21	61:15	First body movement
22a	67:15	Circulation begins through dorsal aorta and vitelline vessels.
22b	71:00	Circulation through ducts of Cuvier
23	74:00	Otoliths develop (first visible concretions).

Table 2 continued...

Table 2 Continued.

Nominal Stage	Developmental time (h:min.) assigned at 25C	Description
24	77:00	Prominent fin buds developed
25	96:00	Retinal pigmentation begins
26	114:00	Liver first appears
27a	132:00	Pectoral fins rounded
27b	138:00	Lens just obscured by retinal pigment
28	144:00	Peritoneal wall pigmented
29	162:00	Circulation in pectoral fins
30	174:00	Rays appear in caudal fin
31a	280:00	Swim bladder appears
31b	310:00	Neural and haemal arches visible in caudal vertebrae
32	440:00	Hatching

Incubation baths were checked for hatches usually once daily, 5 or 6 days per week. During this process, the water in each jar or vial was gently aerated. Fry were removed from the baths within 1 or 2 days of hatching and transferred to covered Petri dishes or 100 ml disposable plastic beakers for rearing at 25C in room 407. If incubated in freshwater, salinity was increased to 40% sea water over a couple of days. Fry were fed *Artemia* nauplii once daily, usually 6 days per week. Fish were usually transferred to 600 ml of water in glass jars after attaining total lengths of about 10 mm or more. Several fish were often reared in the same jar until they approached sexual maturity, when they were segregated into individual jars or aquaria.

2.4 Experimental Treatments

The following experimental series of eggs were collected:

A. 25P25I-FW and -BW.[1]

Eggs in these series were collected from parents at 25C and incubated at 25C, either in freshwater or in 40% sea water. The former series was collected between October 1976 and December 1977, the latter between June 1977 and February 1978. The 25P25I-FW series

[1]Notation in the naming of experimental treatments is as follows: 25P and 30P refer to parental temperatures of 25C and 30C, respectively; 25I and 30I refer similarly to extraparental incubation temperatures; FW and BW refer to extraparental incubation in freshwater and 40% sea water, respectively; FWD refers to extraparental incubation in darkness in freshwater.

provided the 25C sustained incubation temperature control for fish with parents at 25C in temperature break experiments. In addition, both series were used in tests for the effects of the following factors on meristic counts:

1) Duration of intraparental incubation - Duration of intraparental incubation was estimated for each egg as follows:

$$DII = STAGE - HRCS - \tfrac{1}{2}(HRLC) \text{ if } STAGE \geq (HRCS + HRLC)$$

$$DII = (STAGE - HRCS)/2 \text{ if } STAGE < (HRCS + HRLC)$$

where DII = estimated duration (h) of intraparental incubation

STAGE = developmental time (h) at staging (as given in Table 2)

HRCS = time between collection and staging

HRLC = time between collection of an egg and previous check for eggs.

This estimate is obviously only a rough approximation since the actual developmental time within a given nominal stage and the actual time of laying (between collection and the previous check for eggs) were unknown. In both cases, the mid-point of the total possible time interval was used in the estimate (except for some nominal stages for which judgement of an embryo's position within the stage was possible).

2) Parental age and reproductive history - Parental age was approximated as the time in days between the collection of an egg and the collection of its parent as an egg. Parental reproductive history (PRH) is defined as the number of days between the date of collection of an egg and the date of first egg collection from its parent.

3) Grandparent temperature history (GPTH) - Grandparent temperature history refers to the temperature at which an individual's grandparent was held.

4) Date of collection (tested using the FW series only).

5) Incubation salinity - The two 25P25I series were compared to investigate the effect of incubation salinity on meristic counts. This comparison was analyzed as a "salinity break" experiment: fish in the FW series were grouped by developmental stage at transfer to freshwater; the entire BW series was used as a "sustained" brackish water control. This interpretation is obviously a simplification since the salinity experienced during intraparental incubation is probably not exactly 40% sea water. An intraparental incubation salinity near that of the body fluids (about 10 - 15‰ or 26 - 40% sea water in fish (Hoar 1975)) is most likely. A value high in this range is probable since the body fluids of marine and euryhaline teleosts tend toward the higher ion concentrations (Holmes and Donaldson 1969, Hoar 1975) and because an influence of the ambient brackish water on salinity in the oviduct is possible. Thus, viewing the BW series as a sustained salinity control is not likely to introduce serious error into the interpretation of results.

B. 30P30I.

Eggs in this series were collected from parents held at 30C and were incubated in freshwater at 30C. This series was used in tests for effects of parental temperature and as a 30C sustained incubation

temperature control in temperature break experiments.

C. 25P30I.

This series constituted the 25C to 30C break experiment. Eggs were collected from parents at 25C and transferred without acclimation to freshwater at 30C, usually immediately after collection and staging.

D. 30P25I.

This series constituted the reciprocal 30C to 25C break experiment. Eggs were collected from parents held at 30C and transferred to freshwater at 25C immediately after collection and staging.

E. 25P25I-FWD.

Since light intensity experienced during incubation varied somewhat over time, this series was collected to determine whether any meristic series in *R. marmoratus* is labile to influence by light. Eggs were collected from parents held at 25C and, after staging, were incubated in freshwater in darkness (except during brief examinations for newly hatched fry). Light was excluded from the incubating eggs by rearing them in vials placed in jars taped with black electrical tape and covered with a metal lid. Results were analyzed in terms of a "light break" experiment: fish were grouped by developmental stage at transfer to darkness, and their counts

compared to those of fish in the 25P25I-FW series (the control for "sustained" incubation in light). This analysis assumes that the light intensity experienced during intraparental incubation resembles that experienced during extraparental incubation in the 25P25I-FW series. Since adult *R. marmoratus* possess little pigment and are quite translucent, this assumption probably introduces little error into the interpretation of results.

F. Post-hatching thermolability series.

Fish incubated at 30C were transferred to 25C within 1 - 2 days of hatching. Vertebral and pectoral fin ray numbers are fixed in *R. marmoratus* before hatching (Lindsey and Harrington 1972). To test whether other meristic series were also fixed by hatching, fish collected from parents at 25C and incubated in 40% sea water at 25C were transferred to 40% sea water at 30C one or two days after hatching. These fish were held at 30C for several weeks. Their meristic counts were compared to those of fish in the 25P25I-BW series.

2.5 Statistical Procedures

T-tests, performed by the SPSS T-TEST program, were used for most comparisons of meristic counts. Separate variance estimates were used when variances were significantly ($p < .05$) heterogeneous. Simple and multiple linear regressions, polynomial regressions and analyses of covariance were also performed using packaged computer programs (BMDP6R, BMDP5R and BMDPIV, respectively). Effects were assumed to be additive in analyses involving two or more independent variables.

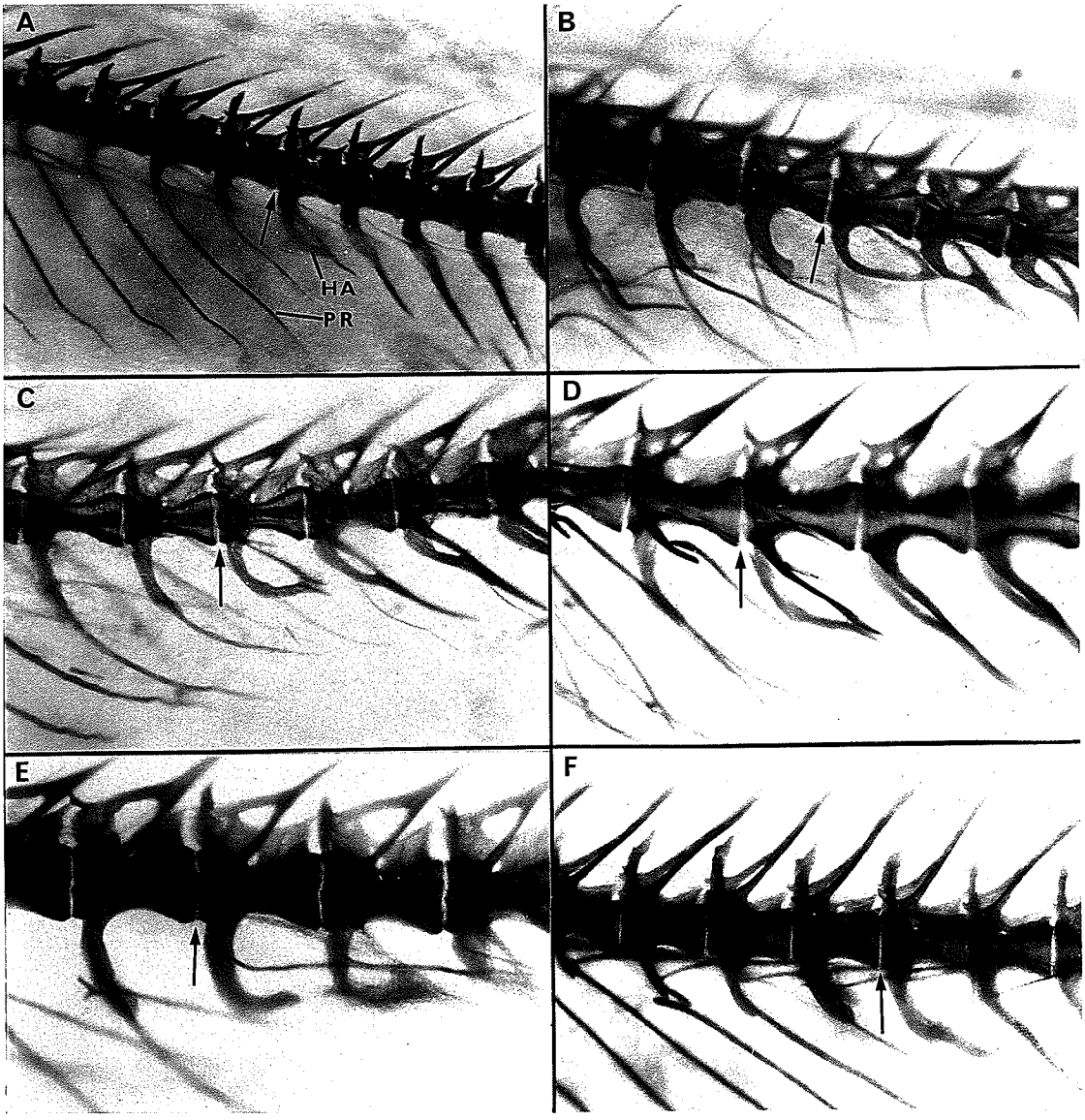
2.6 Staining and Counting Procedures

After reaching total lengths of 15 mm or more, experimental fish were killed, fixed in 5% formalin, cleared in 5% potassium hydroxide, stained with alizarin and transferred to glycerine (procedure modified from Hollister, 1934).

Abdominal, caudal and total vertebrae were counted. Counts included the urostyle. The first caudal vertebra was usually easily distinguished from the last abdominal vertebra by a closed haemal arch and the absence of attached pleural ribs (Figure 1). Occasionally vertebrae intermediate between typical abdominal and caudal vertebrae occurred at the division point. Vertebrae lacking attached pleural ribs but having an incompletely closed haemal arch were classified as caudal vertebrae, as were those having closed haemal arches but bearing minute "ribs" (Figure 1).

Vertebral irregularities were recorded separately according to location in the vertebral column. Four locations were distinguished: the first or post-cranial vertebra, other abdominal vertebrae, the pre-urostyle vertebra, and other caudal vertebrae. After Kandler (1932, 1935), post-cranial and pre-urostyle vertebrae with double neural and/or haemal arches were classified as "complex" vertebrae, while other vertebrae with two or more neural and/or haemal arches were classified as vertebral "fusions". Post-cranial and pre-urostyle vertebrae with more than two neural and/or haemal arches were also classified as fusions.

Figure 1. The division between abdominal and caudal regions of the vertebral column. A. Typical division. B.-F. Divisions involving vertebrae intermediate between typical abdominal and caudal vertebrae. Division points are indicated by arrows. HA = haemal arch; PR = pleural rib.


A
HA
PR
B
C
D
E
F

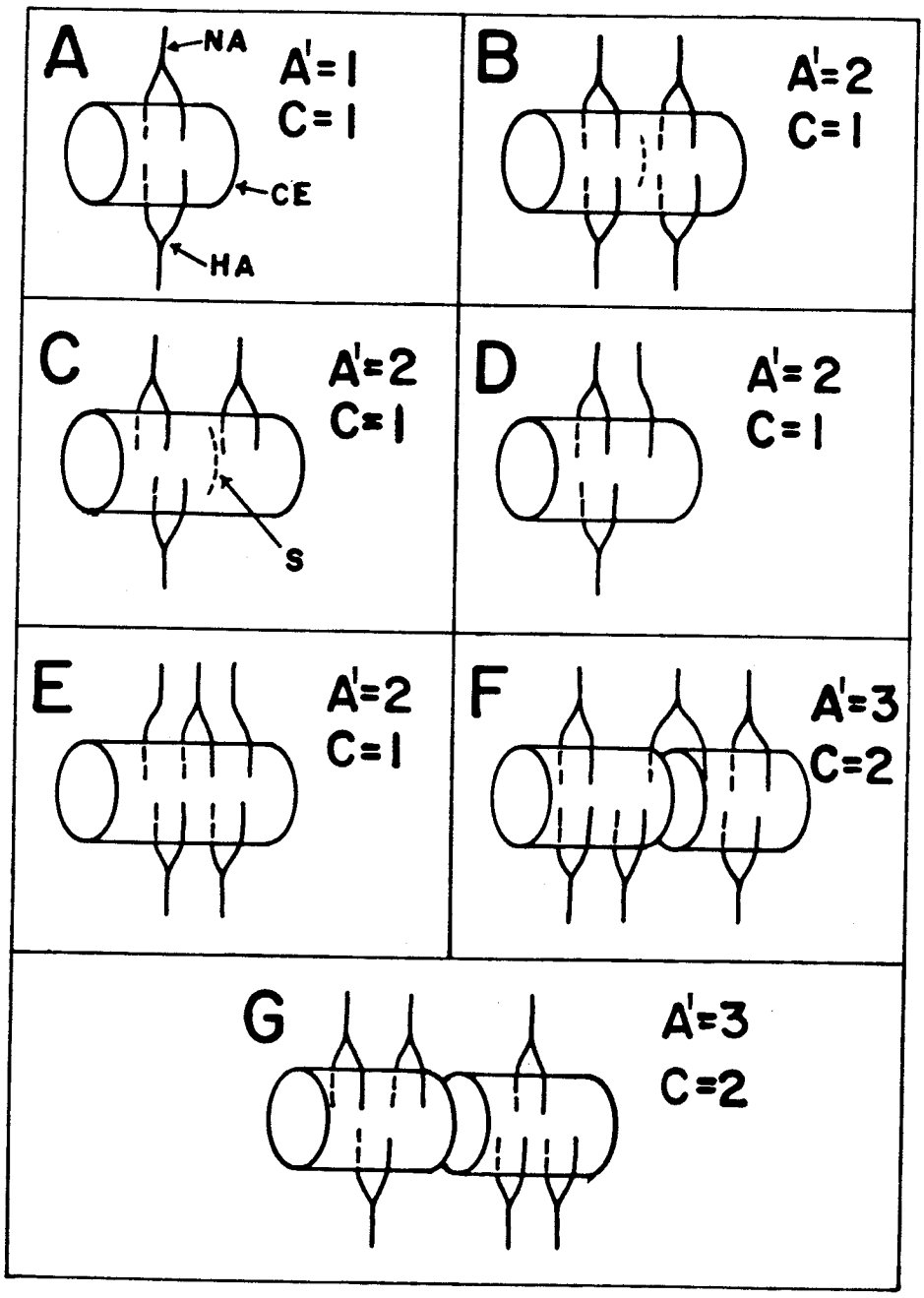
Three indicators of vertebral number were recorded: arch, centrum and "vertebral" counts. Figure 2 summarizes the rules followed in assigning arch and centrum counts. For arch counts, a "vertebra" was assigned a value equal to the number of pairs of neural and haemal arches it bore. If the numbers of neural and haemal arches were unequal, the greater number was used. Extra half arches were counted as one. When arch and centrum formations were out of alignment, a count was obtained by grouping over the series of centra with which the arches were out of alignment, instead of over single centra. For centrum counts, each separate centrum was counted as one, regardless of the number of arches born. "Vertebral" counts were equivalent to arch counts, except that complex pre-urostyle vertebrae were assigned values of 1.5 in the former and 2 in the latter. Reasons for assigning values of 1.5 to complex pre-urostyle vertebrae will be explained in Section 4.1.

Fin ray counts included all elements stained by alizarin, even rudimentary ones. Four types of ray abnormalities were recorded:

1. Short, knob-like rudimentary rays whose heights were less than five times their basal diameters.
2. Short, slab-like rays lacking the typical basal structure.
3. Fused, adjacent rays, distinguished as two rays by separate bases (counted as 2 rays).
4. Rays with bases branching into 3 forks instead of the usual 2 (counted as 1 ray).

Type 1 abnormalities occurred only at the anterior or, rarely, the

Figure 2. Methods of assigning arch and centrum counts. A. Typical vertebra. B. and C. Centra with double haemal and/or neural arches. D. Centrum with extra ½ neural arch. E. Centrum with right and left halves of neural arches out of alignment. F. and G. Centra and arches out of alignment. NA = neural arch; HA = haemal arch; CE = centrum; S = suture line; A^1 = arch count; C = centrum count.


A
NA
A'=1
C=1
CE
HA
B
A'=2
C=1
C
A'=2
C=1
S
D
A'=2
C=1
E
A'=2
C=1
F
A'=3
C=2
G
A'=3
C=2

posterior of a fin. The other types occurred sporadically at any point in a fin. Counts were not recorded if fins were badly split or too poorly stained. Pelvic fin rays were not counted since one or both of these fins were often missing or reduced to an irregular cluster of bones. Occasionally, one or two additional clusters of irregular bones were formed in the region of the pelvic fins. Rarely, a third pelvic fin was present.

3. RESULTS

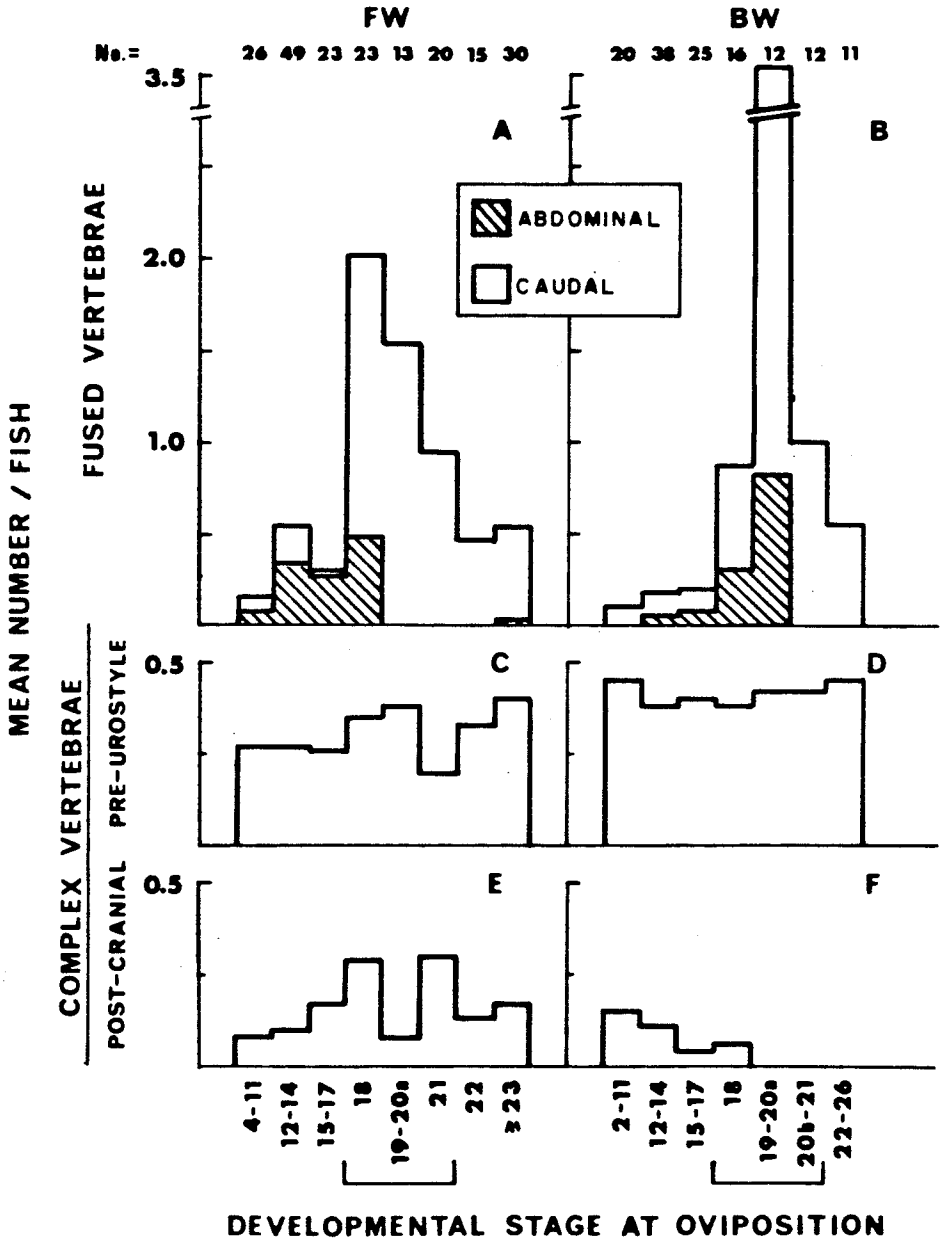
In investigations of parental and incubation temperature effects, which were the main areas of concern in this study, variable egg retention by parents and low individual daily egg production precluded strict control of some extraneous variables among treatments. Eggs contributing to these experiments were collected over a period of many months from parents of varying ages and reproductive histories. Grandparent temperature histories and mean developmental times at oviposition and at transfer to freshwater varied among treatments. Light intensity varied slightly over the collection period. Therefore, before presenting the results of the parental and incubation temperature experiments, the effects of these possible extraneous sources of meristic variation will be described. The occurrence and treatment of vertebral and fin ray irregularities will also be described before presenting the results of parental and incubation temperature experiments.

3.1 Duration of Intraparental Incubation

3.1.1 Vertebrae

The incidence of vertebral fusions showed a striking relation to duration of intraparental incubation in both 25P25I series (Figure 3). Fusions were relatively common in fish laid at stages 18 - 21 but rare in those laid at earlier or later stages. Most fusions were in

Figure 3. Incidence of complex and fused vertebrae in fish grouped by developmental stage at oviposition in the 25P 25I series. Panels A, C and E show incidence in the FW series, panels B, D and F that in the BW series. The total heights of bars in panels A and B denote sums of incidences in the abdominal and caudal regions. Stages at oviposition associated with a high incidence of fused vertebrae are enclosed by brackets for comparison with Figure 4.


FW
BW
No.=
26 49 23 23 13 20 15 30
20 38 25 16 12 12 11
3.5
2.0
1.0
0.5
0.5
A
B
C
D
E
F
ABDOMINAL
CAUDAL
MEAN NUMBER / FISH
FUSED VERTEBRAE
COMPLEX VERTEBRAE
POST-CRANIAL PRE-UROSTYLE
4-11
12-14
15-17
18
19-20a
21
22
≥23
2-11
12-14
15-17
18
19-20a
20b-21
22-26
DEVELOPMENTAL STAGE AT OVIPOSITION

caudal vertebrae; abdominal fusions were rarer and tended to occur most commonly in fish laid over a broader range of intermediate developmental stages (stages 13 - 20). Similar trends occurred in most other experimental series (Table 3). In contrast, the frequencies of complex post-cranial and pre-urostyle vertebrae were not closely related to duration of intraparental incubation (Figure 3).

Unsurprisingly, in both 25P25I series the inclusion of fish with fused vertebrae had a spectacular effect on mean centrum counts but not on mean arch or vertebral counts (Figure 4). However, the few exceptionally low arch and vertebral counts in these series occurred in fish with vertebral fusions and estimated oviposition between stages 17 and 20 (Appendix A).

Slight vertebral variation possibly attributable to duration of intraparental incubation was evident in both 25P25I series even after the exclusion of fish with fused (but not complex) vertebrae from analyses. Although scatter was great (Figure 4), regression analyses indicated that vertebral number tended to decrease slightly with increasing duration of intraparental incubation in both incubation salinities (Table 4). This tendency was most significant in centrum counts in the freshwater series and arch counts in the brackish water series. In both series, this relationship was more significant in subsamples of uniform grandparent temperature history than in the entire samples. The relationship between vertebral number and intraparental incubation time is probably not precisely

TABLE 3. Incidence of fused vertebrae in fish grouped by developmental stage at oviposition in the 25P30I, 30P30I and 25P25I-FWD series.[1]

Nominal Stage at Oviposition	Mean Number of Fused Vertebrae Per Fish								
	25P30I			30P30I			25P25I-FWD		
	N	Abdominal	Caudal	N	Abdominal	Caudal	N	Abdominal	Caudal
2-11	88	.01	.07	6	0	.17	5	0	0
12-14	113	.01	.06	14	0	.21	18	0	0
15-17	39	.20	.41	10	.10	.30	7	.29	1.29
18	30	.17	.60	4	.50	.25	1	0	0
19-20a	11	.09	1.64	7	0	1.14	3	0	.33
20b-21	18	0	.56	3	0	0	3	0	0
22-24	7	0	.14						
≥ 25	25	0	.12	1	0	0			

[1]Post-cranial and pre-urostyle fusions are not included but occurred in 13 out of 439 fish in these series. The 30P25I series is not shown but contained only one fish with a vertebral fusion.

Figure 4. Effects of duration of intraparental incubation on vertebral number in the 25P25I series, including (open circles) and excluding (solid circles) fish with fused vertebrae. Circle areas are proportional to sample sizes. Brackets enclose developmental stages at oviposition which are associated with a high incidence of fused vertebrae. FW and BW denote extraparental incubation in fresh- and brackish water, respectively.

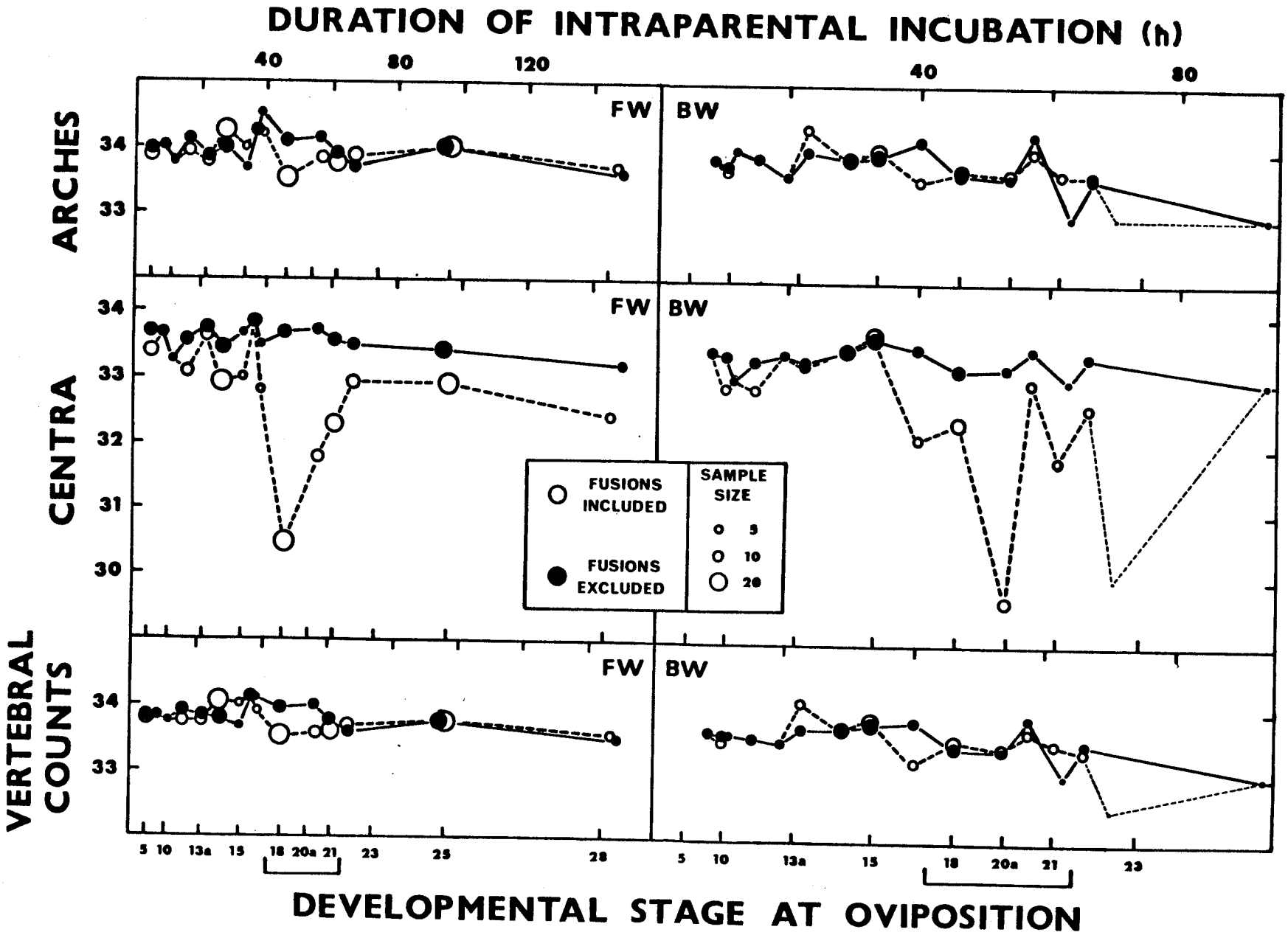

DURATION OF INTRAPARENTAL INCUBATION (h)
40
80
120
40
80
ARCHES
CENTRA
VERTEBRAL COUNTS
FW
BW
34
33
32
31
30
FUSIONS INCLUDED
FUSIONS EXCLUDED
SAMPLE SIZE
5
10
20
5 10 13a 15 18 20a 21 23 25 28
5 10 13a 15 18 20a 21 23
DEVELOPMENTAL STAGE AT OVIPOSITION

TABLE 4. Simple linear regressions between arch, centrum or vertebral counts and estimated duration of intraparental incubation. Fish with fused vertebrae are excluded from analyses.

A. Significance levels and signs of regression slopes for all vertebral series. Solid or broken underlining emphasizes values which are significant at $p \leq .05$ or which approach significance, respectively.

Vertebral series	25P25I-FW		25P25I-BW	
	All fish	Fish with 25C GPTH	All fish	Fish with 30C GPTH
N =	146	92	107	44
Arches, abdominal	0.97 (-)	0.75 (+)	0.43 (-)	1.0
caudal	0.21 (-)	0.13 (-)	0.08 (-)	0.03 (-)
total	0.23 (-)	0.20 (-)	0.05 (-)	0.03 (-)
Centra, abdominal	0.83 (-)	0.70 (+)	0.84 (+)	0.27 (+)
caudal	0.06 (-)	0.01 (-)	0.40 (-)	0.35 (-)
total	0.07 (-)	0.04 (-)	0.43 (-)	0.50 (-)
Total vertebral counts	0.09 (-)	0.04 (-)	0.10 (-)	0.09 (-)

TABLE 4. (Continued...)

B. Least squares regression equations for those vertebral numbers (Y) most strongly associated with duration of intraparental incubation (X).

Series	Sample	Y	Regression Equation	S_b*	r^2†
25P25I-FW	All Fish	Caudal centra	$\hat{Y} = 21.66 - 0.00267\ X$	0.00143	0.024
		Total centra	$\hat{Y} = 33.71 - 0.00287\ X$	0.00159	0.022
		Total vertebral counts	$\hat{Y} = 33.93 - 0.00215\ X$	0.00127	0.019
	Fish with 25C GPTH	Caudal centra	$\hat{Y} = 21.64 - 0.00423\ X$	0.00161	0.071
		Total centra	$\hat{Y} = 33.67 - 0.00380\ X$	0.00178	0.048
		Total vertebral counts	$\hat{Y}$ 33.90 - 0.00289 X	0.00136	0.048
25P25I-BW	All Fish	Caudal arches	$\hat{Y} = 21.92 - 0.00457\ X$	0.00255	0.030
		Total arches	$\hat{Y} = 34.00 - 0.00553\ X$	0.00275	0.037
		Total vertebral counts	$\hat{Y} = 33.77 - 0.00338\ X$	0.00266	0.026
	Fish with 30C GPTH	Caudal arches	$\hat{Y} = 22.18 - 0.00924\ X$	0.00404	0.111
		Total arches	$\hat{Y} = 34.18 - 0.00924\ X$	0.00404	0.111
		Total vertebral counts	$\hat{Y} = 33.86 - 0.00695\ X$	0.00403	0.066

*S_b= standard deviation of the sample regression coefficient

†r^2= coefficient of determination.

linear (see Section 4.4), but no other pattern is discernible in either 25P25I series (Figure 4). In all cases, most vertebral variation related to intraparental incubation time occurred in the caudal region (Table 4). The amount of vertebral variation explained by regression on duration of intraparental incubation was low however (2 - 11%), even in the most significant cases.

3.1.2 Fin Rays

Slight but statistically significant variation related to intraparental incubation time occurred in some fin ray series in both incubation salinities (Table 5). In the whole samples, relationships between fin ray counts and intraparental incubation time were obscured by a tendency for fish with vertebral fusions to have low fin ray counts (Appendix A). After the exclusion of fish with vertebral fusions from analyses, right and average pectoral ray counts displayed significant negative regressions with duration of intraparental incubation in both 25P25I series. The statistical significance of these regressions may however be an artifact of the virtual absence of variation among pectoral ray counts in these series (Appendix A). According to the regression analyses, increasing intraparental incubation time is significantly associated with decreasing caudal ray counts only in the freshwater series, and with increasing anal ray counts only in the brackish water series (Table 5). Despite these apparently significant relationships, scatter among

TABLE 5. Simple linear regressions between fin ray counts and estimated duration of intraparental incubation.

A. Significance levels (p) and signs of regression slopes for all fin ray series. Significant values ($p \leq .05$) are underlined.

	25P25I-FW				25P25I-BW			
	All fish		Excluding fish with vertebral fusions		All fish		Excluding fish with vertebral fusions	
Fin	p	N	p	N	p	N	p	N
Pectoral, right	<u>0.0001</u> (-)	197	<u>.01</u> (-)	141	0.15 (-)	94	<u>0.01</u> (-)	73
left	<u>0.03</u> (-)	200	.26 (-)	144	0.94 (+)	93	0.32 (-)	73
average			<u>.04</u> (-)	141			<u>0.01</u> (-)	73
Dorsal	<u>0.05</u> (-)	198	.21 (-)	143	<u>0.004</u> (-)	97	0.34 (-)	75
Anal	0.16 (-)	179	.15 (-)	128	0.15 (+)	93	<u>0.02</u> (+)	71
Caudal	<u>0.004</u> (-)	199	<u>.04</u> (-)	143	0.52 (+)	98	0.38 (+)	75

B. Least squares regression equations for those fin ray counts (Y) most significantly associated with duration of intraparental incubation (X) in samples excluding fish with vertebral fusions.

Series	Y	Regression Equation	S_b*	r^2†
25P25I-FW	Right pectoral rays	$\hat{Y} = 14.04 - 0.00114X$	0.00046	0.043
	Average pectoral rays	$\hat{Y} = 14.05 - 0.00091X$	0.00042	0.032
	Caudal rays	$\hat{Y} = 30.85 - 0.00460X$	0.00221	0.030
25P25I-BW	Right pectoral rays	$\hat{Y} = 14.07 - 0.00475X$	0.00183	0.087
	Average pectoral rays	$\hat{Y} = 14.04 - 0.00339X$	0.00127	0.092
	Anal rays	$\hat{Y} = 11.22 - 0.01057X$	0.00441	0.077

*S_b = standard deviation of the sample regression coefficient

†r^2 = coefficient of determination.

mean caudal and anal ray counts of fish grouped by estimated duration of intraparental incubation was great, and no clear relationships were evident (Appendix A). Departures from truly linear relationships are likely (see Section 4.4). Dorsal ray counts were apparently unrelated to intraparental incubation time (Table 5).

Developmental time at oviposition was highly correlated with time at transfer to freshwater in the freshwater series ($r^2 = 0.96$). The close association between oviposition and an appreciable salinity break in the freshwater but not in the brackish water series is the probable source of apparent differences in the relationships between fin ray counts and duration of intraparental incubation between the two series. The effect of transfer to freshwater evidently tended to cancel the effect of oviposition on anal ray counts in the freshwater series. The significant regression between caudal ray count and intraparental incubation time in the freshwater series is apparently entirely due to transfer to freshwater during development.

3.2 Occurrence and Treatment of Meristic Irregularities

3.2.1 Fused and Complex Vertebrae

Table 6 shows the distribution of fused and complex vertebrae among treatments. Abdominal fusions were rare in all treatments. Variation in the incidence of caudal fusions roughly reflected variation in the percentage of eggs laid between stages 17 and 21 in each treatment. The incidence of complex vertebrae varied widely among

TABLE 6. Frequency (%) of fish with fused or complex vertebrae in each major experimental series.

Treatment	N	% laid at stages 17-21	Post-cranial		Other Abdominal Fusions (No./fish)							Pre-urostyle							Other Caudal Fusions (No./fish)							
			Complex	Fusions(No.) 2	1	2	3	4	5	9	Total	Complex	Fusions (No.) 2	3	4	7	8	Total	1	2	3	4	5	6	7	Total
30P30I	50	42	8.0	0	2.0	4.0	0	0	0	0	6.0	46.0	0	4.0	0	0	2.0	6.0	14.0	6.0	0	0	0	2.0	0	22.0
25P25I-BW	147-148*	34	6.1	0.7	0.7	1.4	0.7	0.7	0	0.7	4.2	40.5	2.0	0	0.7	0.7	0	3.4	9.5	6.1	0.7	2.0	0.7	0.7	0.7	20.4
25P25I-FW	208-209*	31	17.2	1.0	3.3	3.3	0.5	0	1.0	0	8.1	30.3	1.4	0.5	0	0	0	1.9	8.7	6.3	2.9	2.4	1.0	1.4	0	22.7
25P25I-FWD	37	22	10.8	0	5.4	0	0	0	0	0	5.4	16.2	0	0	0	0	0	0	2.7	0	2.7	0	0	2.7	0	8.1
25P30I	350-352*	20	19.7	0.6	1.4	0.6	0.3	0.3	0	0	2.6	25.6	1.4	0.9	0	0	0	2.3	3.7	3.1	1.7	1.4	0.6	0	0	10.5
30P25I	75	16	8.0	0	1.3	0	0	0	0	0	1.3	20.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

*1 or 2 fish are omitted from some tabulations due to damage or poor staining in restricted regions of the vertebral column.

treatments. Complex pre-urostyle vertebrae were the most common type of vertebral irregularity in all treatments.

Complex and fused vertebrae differed greatly in morphology. Vertebral fusions usually involved considerable irregularity and distortion of arches and centra and generally showed at least a trace of a suture (Figure 5). In contrast, complex post-cranial vertebrae usually lacked any indication of a suture and almost invariably displayed no irregularity, except doubled neural arches and transverse processes and about twice the usual length (Figure 6). Complex pre-urostyle vertebrae also usually lacked any marked irregularity (Figure 7). Unlike post-cranial vertebrae, pre-urostyle vertebrae appeared to display a complete range of variation from single to almost completely doubled vertebrae (Figure 7). (Pre-urostyle vertebrae were recorded as "complex" only if separation of neural and/or haemal spines was distinct.).

Their morphology and distributions among and within treatments suggest that fused vertebrae may be developmental abnormalities associated with laying at certain stages, while complex pre-urostyle (and possibly post-cranial) vertebrae appear to be responses to treatment (see Section 4.1). Most of the few exceptionally low (or high) meristic counts reported in the previous section occurred in fish with fused vertebrae. In the two 25P25I series, fish with vertebral fusions had significantly lower mean counts and significantly higher variances than did those without fusions for most meristic series (Appendix B).

Figure 5. Caudal portion of a vertebral column with fused vertebrae.

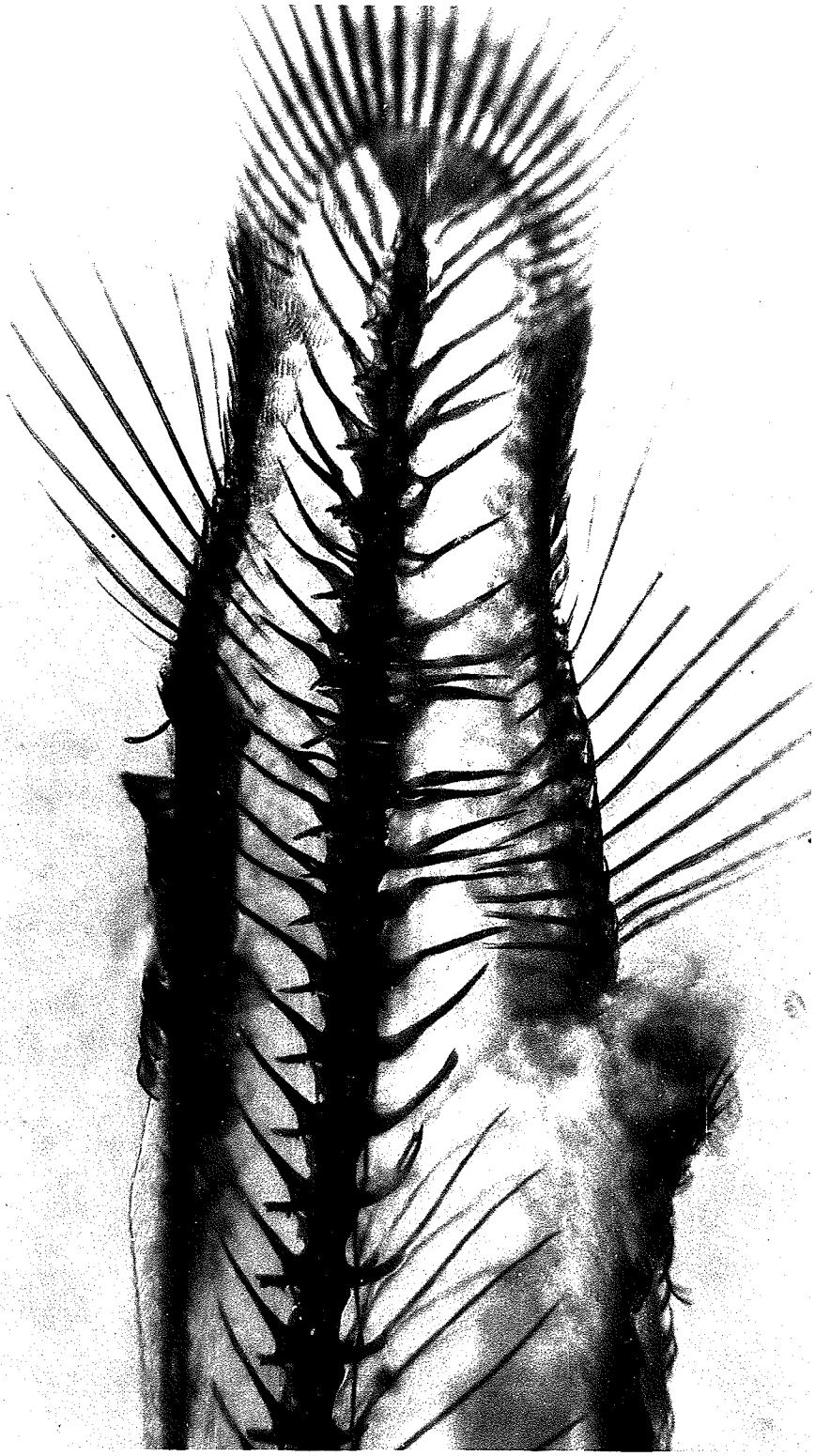


Figure 6. Normal (A,B) and complex (C,D) post-cranial vertebrae (PCV). B. and D. Left lateral views. A. and C. ventral views.

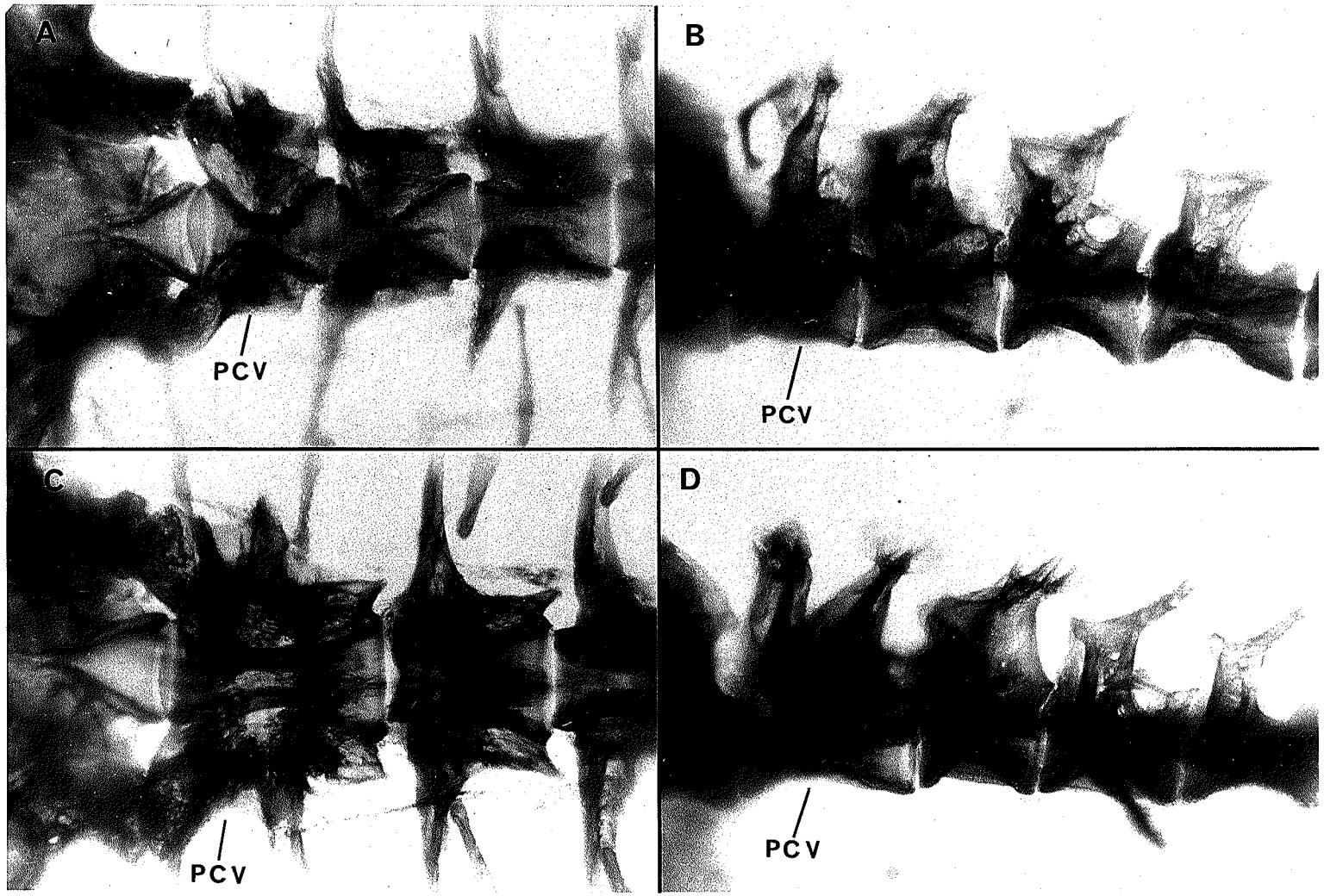
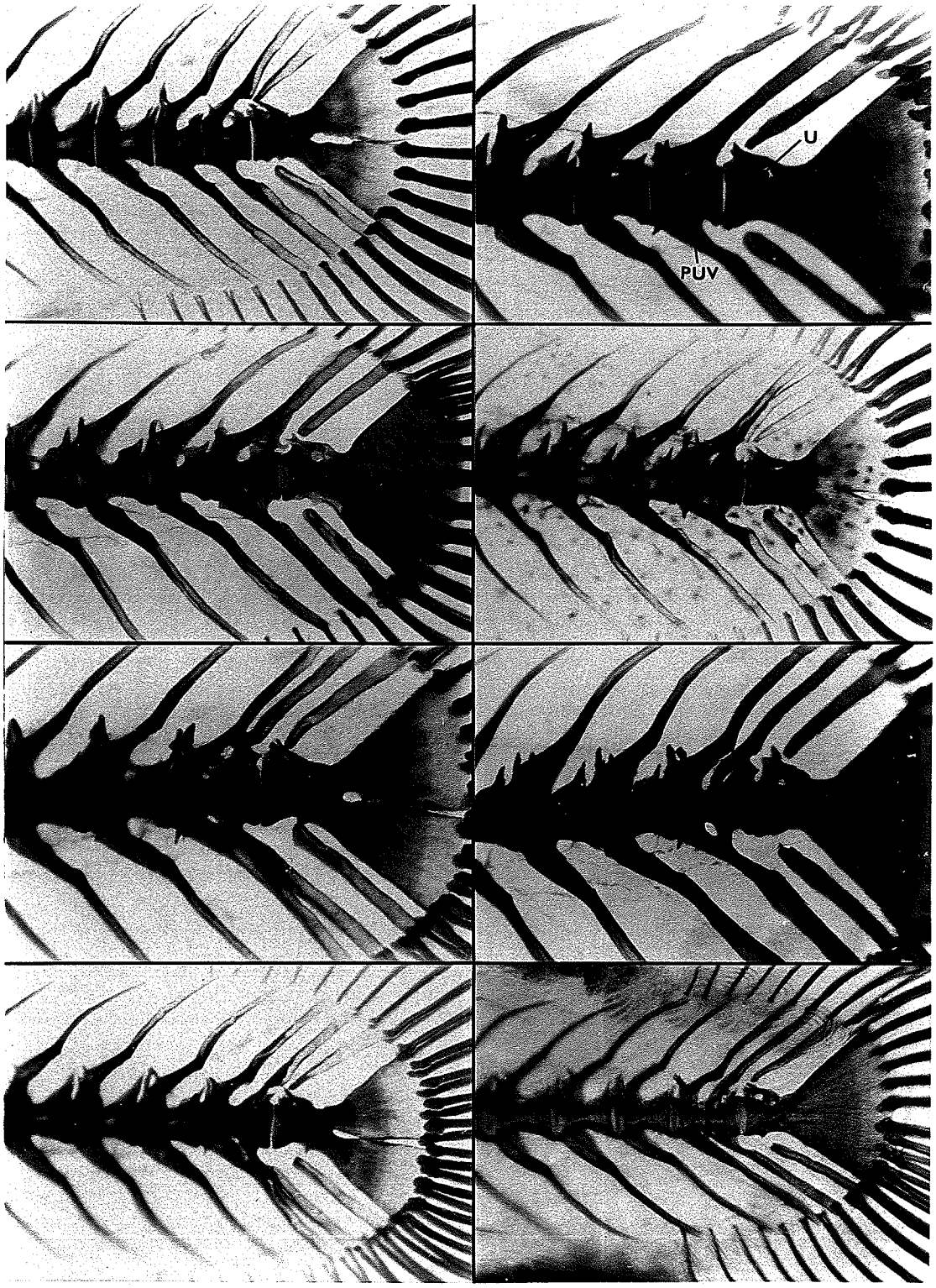

A
PCV
B
PCV
C
PCV
D
PCV

Figure 7. Morphological variation in the pre-urostyle vertebra (PUV). U = urostyle.


U
PUV
PUV

The low mean counts and high variances associated with fish having vertebral fusions could obscure the effects of experimental treatments. For this reason and since their occurrence did not seem to be causally related to any experimental treatment, fish with vertebral fusions (about 15 - 20% of the total sample) were excluded from all analyses reported below. Fish with complex post-cranial or pre-urosytle vertebrae were included in all analyses.

3.2.2 Fin Ray Abnormalities

Fin ray abnormalities were rare in all fins in all experimental treatments, occurring in only 1 - 4% of the fish in the total sample (Appendix C). Counts of fins with abnormal rays were not excluded from analyses.

3.3 Parental Age and Reproductive History

3.3.1 Parental Reproductive History (PRH)

The influence of parental reproductive history on meristic counts was examined in the two 25P25I series.[1] Because this influence appeared to be obscured by confounding with grandparent temperature history (GPTH) in some meristic series, results are presented both

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[1] Fin ray counts are not presented for the brackish water series because poor staining rendered the rays of most fish laid soon after the onset of oviposition uncountable in this series.

for the whole samples and for subsamples of uniform GPTH (provided the latter are of sufficient size and range of PRH[1]).

Both in the fresh- and brackish water series, offspring of parents which had just begun to lay eggs tended to have fewer meristic parts than did those of "experienced" parents (Figure 8 and 9). The statistical significance of this tendency, assessed using t-tests and simple linear regression analyses, is shown in Table 7 for each meristic series.

In the freshwater series, the effect of parental reproductive history was most significant for anal and dorsal rays and non-significant for caudal rays (Table 7). In most cases, left but not right pectoral ray counts were significantly related to parental reproductive history (Table 7); however, the magnitude of this effect was similarly slight for both left and right pectoral fins (Figure 8).

Among vertebral series, an influence of parental reproductive history was most apparent in the centrum counts of fish incubated in freshwater and in the arch counts of those reared in brackish water (Table 7, Figure 9). Most vertebral variation related to parental reproductive history occurred in the caudal region (Table 7). In some samples, numbers of abdominal vertebrae tended to decrease slightly among fish produced long after the onset of oviposition.

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[1] Separate analyses are not presented for the subsample of known 30C GPTH in the freshwater series due to its small sample size, nor for that of known 25C GPTH in the brackish water series due to an insufficient range of PRH.

Figure 8. Effects of parental reproductive history on fin ray counts in the 25P25I-FW series. Circle areas are proportional to sample sizes. Vertical lines (solid for fish with 25C GPTH, broken for fish with unknown GPTH) show 95% confidence intervals.

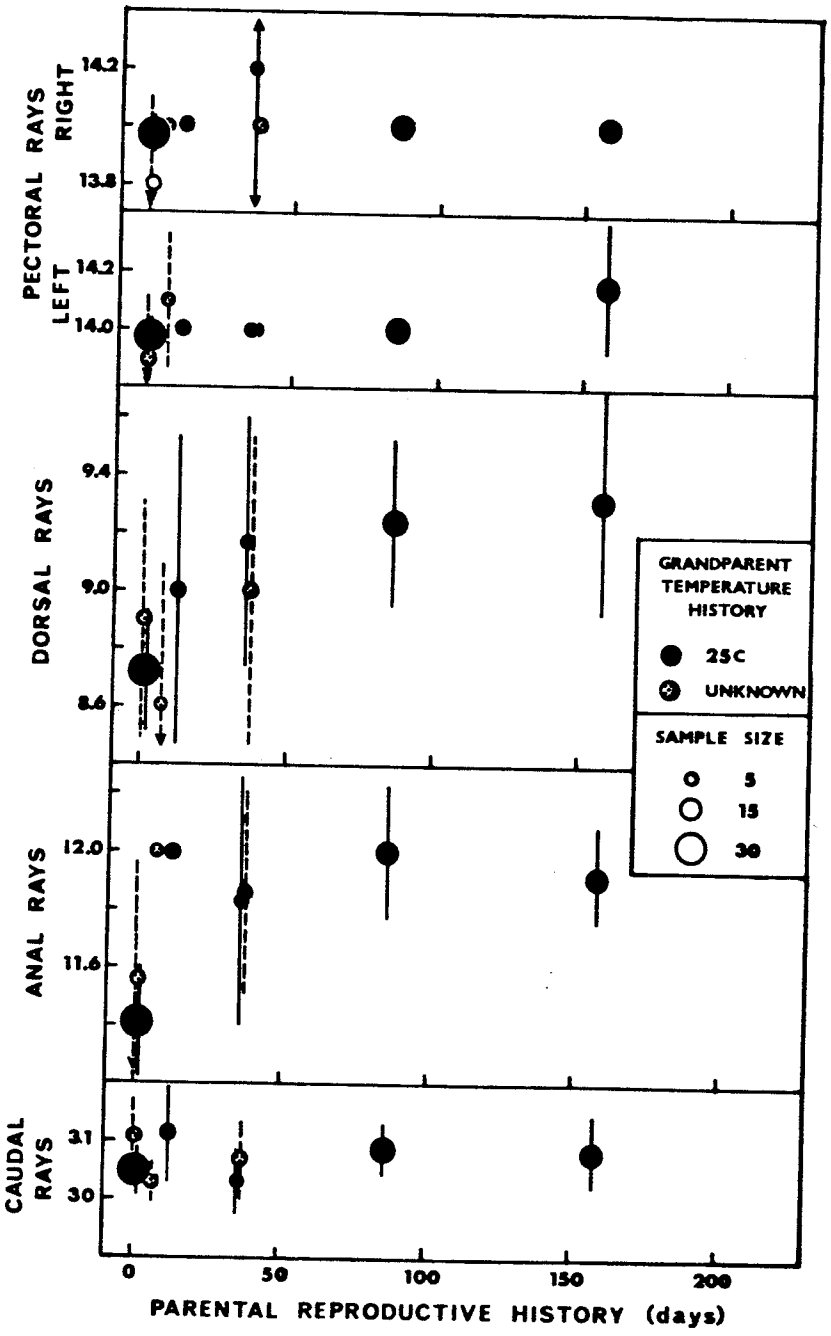

PECTORAL RAYS
RIGHT
14.2
13.8
LEFT
14.2
14.0
DORSAL RAYS
9.4
9.0
8.6
ANAL RAYS
12.0
11.6
CAUDAL RAYS
31
30
GRANDPARENT TEMPERATURE HISTORY
25C
UNKNOWN
SAMPLE SIZE
5
15
30
0
50
100
150
200
PARENTAL REPRODUCTIVE HISTORY (days)

Figure 9. Effects of parental reproductive history on vertebral number in the 25P25I series. Circle areas are proportional to sample sizes. Vertical lines (solid for fish with 25C GPTH, broken for fish with unknown or 30C GPTH) show 95% confidence intervals. FW and BW denote extraparental incubation in fresh- and brackish water, respectively.

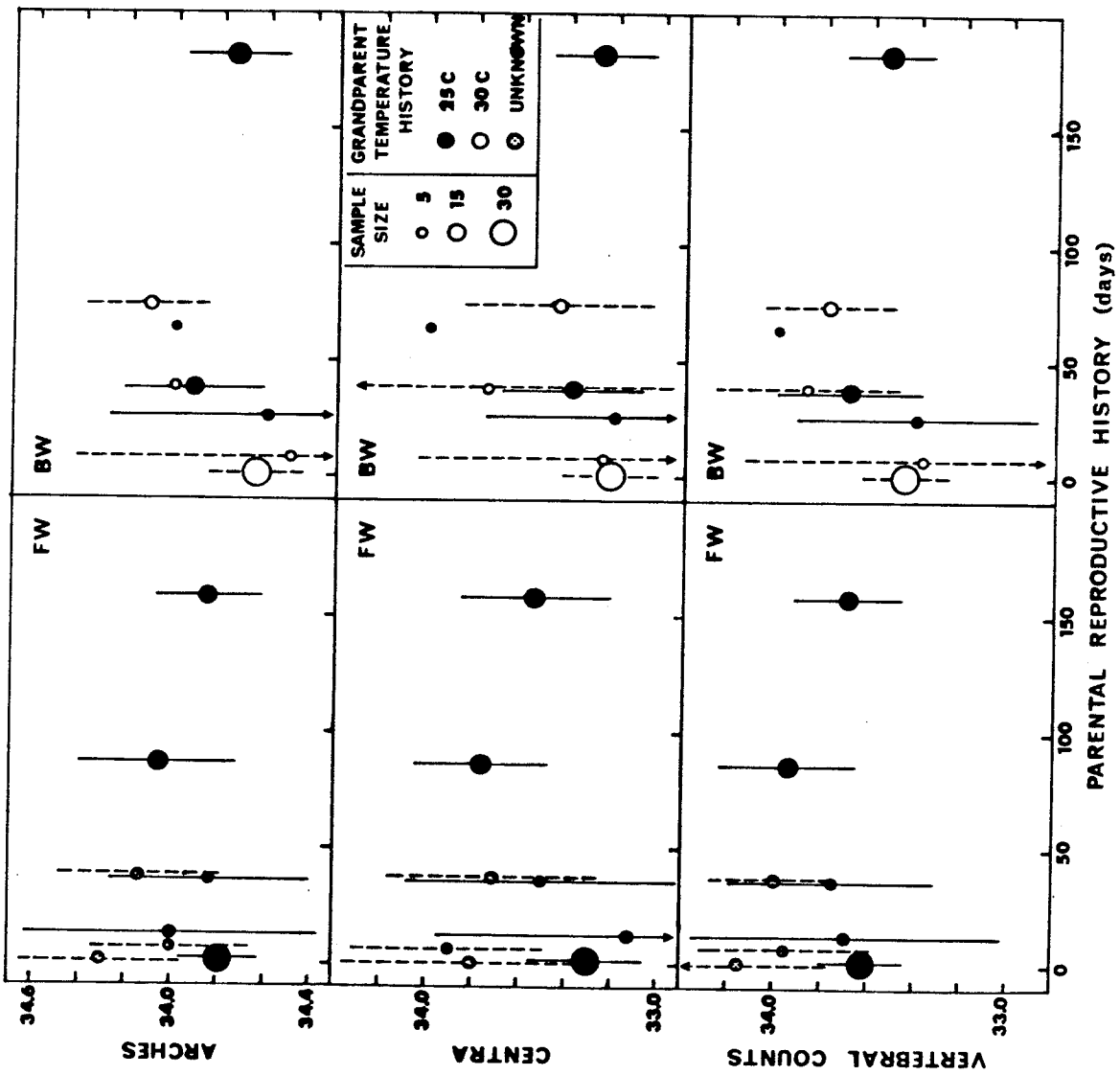

ARCHES
CENTRA
VERTEBRAL COUNTS
34.6
34.0
34.4
34.0
33.0
34.0
33.0
FW
BW
SAMPLE SIZE
3
15
30
GRANDPARENT TEMPERATURE HISTORY
25 C
30 C
UNKNOWN
0
50
100
150
PARENTAL REPRODUCTIVE HISTORY (days)

TABLE 7. Significance levels in tests for an effect of parental reproductive history on meristic counts. The signs of regression slopes are shown after the probability values of regressions. The most significant values are emphasized as in Table 4. Regression equations are given in Appendix D.

Meristic Series	FRESHWATER				BRACKISH WATER			
	All Fish		25C GPTH		All Fish		30C GPTH	
	T-test[1]	Linear Regression	T-test[1]	Linear Regression	T-test[1]	Linear Regression	T-test[1]	Linear Regression
Vertebral Arches:								
Abdominal	.32	.19(-)	.16	.09(-)	.35	.49(-)	.56	.57(-)
Caudal	.38	.58(+)	.02	.05(+)	.03	.44(+)	<.001	.006(+)
Total	.83	.79(-)	.23	.45(+)	.01	.68(+)	.001	.01(+)
Vertebral Centra:								
Abdominal	.75	.82(+)	.70	.89(-)	.73	.06(-)	1.0	.94(-)
Caudal	.16	.56(+)	.01	.05(+)	.07	.42(+)	.10	.15(+)
Total	.12	.52(+)	.03	.07(+)	.17	.97(+)	.10	.16(+)
Total Vertebral Counts	.51	.84(-)	.10	.32(+)	.01	.61(+)	.008	.04(+)
Fin Rays								
Average Pectoral		.006(+)		.006(+)				
Right Pectoral	.05	.27(+)	.16	.62(+)				
Left Pectoral	.05	<.001(+)	.09	<.001(+)				
Dorsal	.01	.005(+)	<.001	.006(+)				
Anal	<.001	.001(+)	<.001	<.001(+)				
Caudal	.74	.97(+)	.21	.56(+)				

[1]T-tests were between fish laid less than 5 days vs. more than 30 days after the onset of oviposition.

Thus, within subdivisions of the vertebral column, some of the variation related to parental reproductive history may have been due to shifts in the abdominal-caudal division of the column.

The effect of parental reproductive history on vertebral number appeared to be obscured in the whole samples by confounding with grandparent temperature history (GPTH) or some related variable (Table 7). In the freshwater series, vertebral differences related to PRH were significant only in the subsample of known 25C grandparentage. In this series, fish of unknown (and possibly 30C) grandparentage tended to have high vertebral counts and short PRH (Figure 9), thereby obscuring the effect of PRH in the whole sample. In the brackish water series, some vertebral differences related to PRH were significiant in the whole sample, but levels of significance were generally greater in the subsample of known 30C grandparentage. Fish with grandparents held at 25C tended to have low vertebral counts and moderate to long PRH (Figure 9), accounting for the reduced significance levels in the whole brackish water sample.

Although linear regressions between counts and parental reproductive histories were significant in many cases, meristic responses to PRH were probably not actually linear over the entire range of histories used in this study. In most series, numbers of parts appeared to peak or plateau at or before PRH of 100 days (Figures 8 and 9). Vertebral counts actually tended to decrease slightly at very long PRH. These decreases were associated with relatively long intra-

parental incubation (Table 8), but persisted even after adjustment of counts to a constant intraparental incubation time using regression techniques (not shown). Quadratic and cubic regressions provided statistically superior fits for some fin ray series (Appendix D), but predicted very unrealistic counts at long PRH and were accordingly not used when adjustments to a constant PRH were required in analyses below.

Harrington (1963) reported that older fish tend to lay eggs at relatively earlier embryonic stages. Since vertebral and possibly pectoral ray counts increase slightly with decreasing intraparental incubation time, increases in these counts with increasing PRH could be spurious effects of concommittant decreases in duration of intraparental incubation. However, intraparental incubation time and PRH were not significantly correlated in any subsample of uniform GPTH (Table 8). Multiple linear regressions with intraparental incubation time and PRH as independent variables confirmed the independent effect of each on vertebral and pectoral ray numbers (Appendix D).

In summary, parental reproductive history significantly influences most meristic counts. Whether this influence is due to the intraparental incubation environment, or to an earlier prefertilization effect, cannot be conclusively decided from available data (but the latter possibility is suggested by some arguments presented in Appendix E).

TABLE 8. Mean duration of intraparental incubation (h) of fish grouped by parental reproductive history in the 25P25I series. Sample sizes shown in parentheses.

Sample	Parental Reproductive History (days)							p*
	0-4	5-10	11-15	16-30	31-60	61-100	>100	
FW series; 25C GPTH	58.7 (30)	9.8 (2)	50.5 (6)	38.4 (2)	19.7 (4)	41.1 (17)	62.1 (13)	0.94
BW series; 25C GPTH	31.1 (1)	-	23.2 (2)	27.2 (5)	12.9 (13)	9.5 (3)	27.2 (19)	0.12
BW series; 30C GPTH	43.8 (27)	53.8 (4)	-	-	30.8 (4)	44.9 (9)	-	0.83

* p = significance level of correlations between duration of intraparental incubation and parental reproductive history.

3.3.2 Parental Age

Unsurprisingly, parental age and reproductive history were highly correlated ($p < .01$). Meristic series responded similarly to both variables in most groups of fish, with one exception. The development of one group of parents was apparently stunted by crowded conditions experienced until the onset of breeding. These parents began egg laying at an unusually old age. Offspring laid by these old fish soon after the onset of breeding were compared to those from younger parents with longer reproductive histories. Thus, in these comparisons, the correlation between parental age and reproductive history was negative instead of positive, the usual case. Meristic series responded in the usual manner to differences in parental reproductive history but in the opposite to the usual manner to differences in parental age. This suggests that the causal agent of the meristic responses is not the age of the parent, but rather the time since the parent started to lay eggs. Hence, meristic responses to variation in parental age will not be reported in more detail.

3.4 Grandparent Temperature History (GPTH)

The preceding analyses suggested an effect of grandparent temperature on numbers of caudal and total vertebrae. Regressions between arch, centrum or vertebral counts and intraparental incubation time were more significant in subsamples of uniform GPTH than in samples of mixed GPTH, despite decreases in sample sizes in the former.

In both 25P25I series, the influence of PRH on vertebral number appeared to be obscured by confounding with GPTH in the entire samples but was highly significant in subsamples of uniform GPTH. The analysis of PRH effects suggested that fish of 30C grandparentage tend to have more vertebrae than those of 25C grandparentage.

Only the brackish water series contained enough fish of known 25C and 30C grandparentage to directly test for an effect of GPTH on meristic counts. Comparisons are hampered by confounding with intraparental incubation time and PRH (Table 8). Vertebral differences between fish of 25C and 30C grandparentage cannot be attributed to confounding with the former: the longer average intraparental incubation of fish with grandparents held at 30C should tend to obscure the apparent effect of GPTH on vertebral number. Confounding with PRH is a more serious obstacle to comparisons between the two GPTH groups.

In attempts to correct for confounding with PRH, meristic counts of fish with 25C and 30C grandparent temperatures were compared in three ways:

1) Vertebral counts in the 30C GPTH subsample were adjusted to those expected at the mean PRH of the 25C GPTH group (99.8 days), using linear regression techniques. After this adjustment, highly significant differences in numbers of caudal and total arches and in total vertebral counts occurred between the two GPTH groups (Table 9A). However, this comparison is not valid if vertebral responses to PRH are

TABLE 9. Comparisons of meristic counts of fish in the 25P25I-BW series with grandparents held at 25C or 30C. The most significant probabilities (p) are emphasized as in Table 4.

A. Comparisons of vertebral numbers using entire samples. Counts of fish with 30C grandparentage are adjusted to PRH = 99.8 days, the mean PRH in the 25C GPTH sample, using regression coefficients.*

Meristic Series	30C GPTH			25C GPTH			p
	Mean	S.D.	N	Mean	S.D.	N	
Caudal arches	22.31	0.49	54	21.74	0.44	43	**<0.001**
Total arches	34.30	0.53	54	33.82	0.45	44	**<0.001**
Total vertebral counts	33.97	0.51	54	33.61	0.43	44	**<0.001**

*Adjustment of counts in the 30C GPTH sample were made as follows:

for Y = caudal arches $Ya_i = Y_i + 0.00702\ (99.8-X_i)$
Y = total arches $Ya_i = Y_i + 0.00664\ (99.8-X_i)$
Y = total vertebral counts $Ya_i = Y_i + 0.00525\ (99.8-X_i)$

where X_i = PRH (days)
Y_i = unadjusted count
Ya_i = adjusted count

TABLE 9 (Continued)

B. Comparisons using fish with PRH > 30 days.

Sample	Statistic	Arches			Centra			Total Vertebral Counts	Fin Rays				
		Abdominal	Caudal	Total	Abdominal	Caudal	Total		Pectoral Right	Pectoral Left	Dorsal	Anal	Caudal
30C GPTH	Mean	12.00	22.08	34.08	12.00	21.54	33.54	33.81	13.92	14.00	8.92	11.92	30.00
	S.D.	0	0.28	0.28	0	0.52	0.52	0.32	0.28	0	0.49	0.76	0.41
	N	13	13	13	13	13	13	13	13	13	13	13	13
25C GPTH	Mean	12.06	21.77	33.83	12.00	21.34	33.36	33.62	13.94	13.91	8.80	11.39	30.06
	S.D.	0.24	0.43	0.45	0.24	0.54	0.49	0.44	0.24	0.29	0.63	0.56	0.94
	N	35	35	36	35	35	36	36	33	33	35	31	35
	p=	0.39	0.02	0.07	1.0	0.26	0.27	0.18	0.84	0.27	0.53	0.01	0.77

TABLE 9 (Continued)

C. Comparisons using fish with PRH < 100 days and adjusting for differences in PRH and duration of intraparental incubation using analysis of covariance.

Meristic Series	GPTH (C)	N	Mean count Unadjusted	Mean count Adjusted	$p(Ya)^1$	$p(b=0)^2$	$p(b_{25}=b_{30})^3$
Caudal arches	30	44	21.77	21.87	0.04	0.01	0.14
	25	23	21.74	21.55			
Total arches	30	44	33.77	33.87	0.27	0.01	0.10
	25	24	33.87	33.70			
Total vertebral counts	30	44	33.56	33.63	0.66	0.07	0.44
	25	24	33.69	33.56			

[1] p(Ya) is the probability that adjusted means are the same in the 25C and 30C GPTH samples.

[2] p(b=0) is the probability that regression slopes equal zero.

[3] $p(b_{25}=b_{30})$ is the probability that regression slopes are the same in the 25C and 30C GPTH samples.

concave or asymptotic and level off before 99.8 days of PRH.

2) The two GPTH groups were compared using only fish laid more than 30 days after the onset of oviposition (Table 9B). Using samples so selected, fish of 30C grandparentage had significantly more caudal arches and anal fin rays than did those of 25C grandparentage; the increase in total arch counts in the 30C GPTH subsample approached significance. These differences cannot be attributed to confounding with PRH unless meristic counts are related to PRH in a concave manner.

3) Vertebral and arch counts of fish with 25C or 30C GPTH and PRH of less than 100 days were compared after adjusting for differences in PRH and intraparental incubation time using analysis of covariance (Table 9C). This procedure is probably valid since the relationship between vertebral number and PRH appears to be approximately linear between PRH of 0 and 100 days (Figure 9). For all vertebral series tested, the adjusted mean numbers of vertebrae were greater among fish with grandparents held at 30C, but this difference was significant only for caudal arches.

In summary, all three methods of analysis suggest similar effects of grandparent temperature on numbers of vertebrae. However, most vertebral differences attributed to an influence of grandparent temperature might have resulted from confounding with PRH if the relation between vertebral number and PRH is concave, rather than linear or asymptotic. The failure to detect significant effects of PRH on vertebral number in samples of mixed GPTH might also

have resulted from a strongly concave relation between vertebral number and PRH in the brackish water series, and from a failure to record the onset of oviposition in the parents of fish with unknown GPTH in the freshwater sample. Since the latter is unlikely and there is little indication of strongly concave vertebral responses to PRH, an effect of grandparent temperature on vertebral number seems probable.

3.5 Incubation Salinity

Figure 10 compares meristic counts of fish transferred to freshwater during development with those of fish with "sustained" brackish water incubation. To correct for wide differences in average PRH among transfer lots, dorsal, anal and pectoral ray counts shown in Figure 10 are adjusted to the mean PRH of the brackish water control using linear regression coefficients. Vertebral counts are not adjusted in this figure because the regression between vertebral counts and PRH is not significant in the freshwater series. Moreover, subsampling to obtain uniform GPTH and reduce confounding with PRH revealed that the pattern of vertebral response shown in Figure 10 is not an artifact of confounding with either variable.

To distinguish between the influences of oviposition and of transfer to freshwater, effects of stage at oviposition not related to salinity breaks were assumed to be the same in both fresh- and brackish water series; meristic counts were adjusted using the

significant regressions with intraparental incubation time in the latter series. In Figure 10, anal ray counts were adjusted to the mean duration of intraparental incubation in the brackish water control. Brackish water controls adjusted to mean intraparental incubation times in each transfer lot are plotted for vertebral and pectoral ray counts in this figure (counts of fish in each transfer lot were not adjusted because regressions between vertebral or pectoral ray count and intraparental incubation time were only marginally significant or of doubtful validity). Dorsal and caudal ray counts were not adjusted, since they are not related to intraparental incubation time in the brackish water series. No adjustments were made at intraparental incubation times over 70 h, the maximum value used to establish the regressions in the brackish water series.

All meristic counts were increased by transfer to freshwater at some developmental stages (Figure 10). Comparisons using unadjusted counts (with broader grouping by transfer stage and subsampling to reduce confounding with PRH, GPTH and intraparental incubation time) revealed that these increases were highly significant for all series except pectoral rays, and were not artifacts of inaccurate regression adjustments (Appendix F). Increases in pectoral ray counts were significant in the left fin and approached significance in the right fin in comparisons using all fish regardless of stage at transfer (Appendix F). Patterns and magnitudes of response to salinity

Figure 10. Meristic counts produced by transfers from brackish to freshwater at indicated developmental times. Vertical lines indicate 95% confidence intervals. Circle areas are proportional to sample sizes (see panel C).

A. Dorsal rays. Counts are adjusted to the mean PRH of the brackish water control as follows:

$Y_i(adj) = Y_i + 0.00311\ (84.2 - PRH_i)$.

B. Anal rays. Counts are adjusted to the mean intraparental incubation time (IIT) and/or PRH of the brackish water control as follows:

i) solid circles and lines: $Y_i\ (adj)_1 = Y_i + 0.00266\ X\ (84.2-PRH_i)$

ii) open circles, broken lines: $Y_i\ (adj)_2 = Y_i\ (adj)_1 + 0.01057\ (27.9 - IIT_i)$.

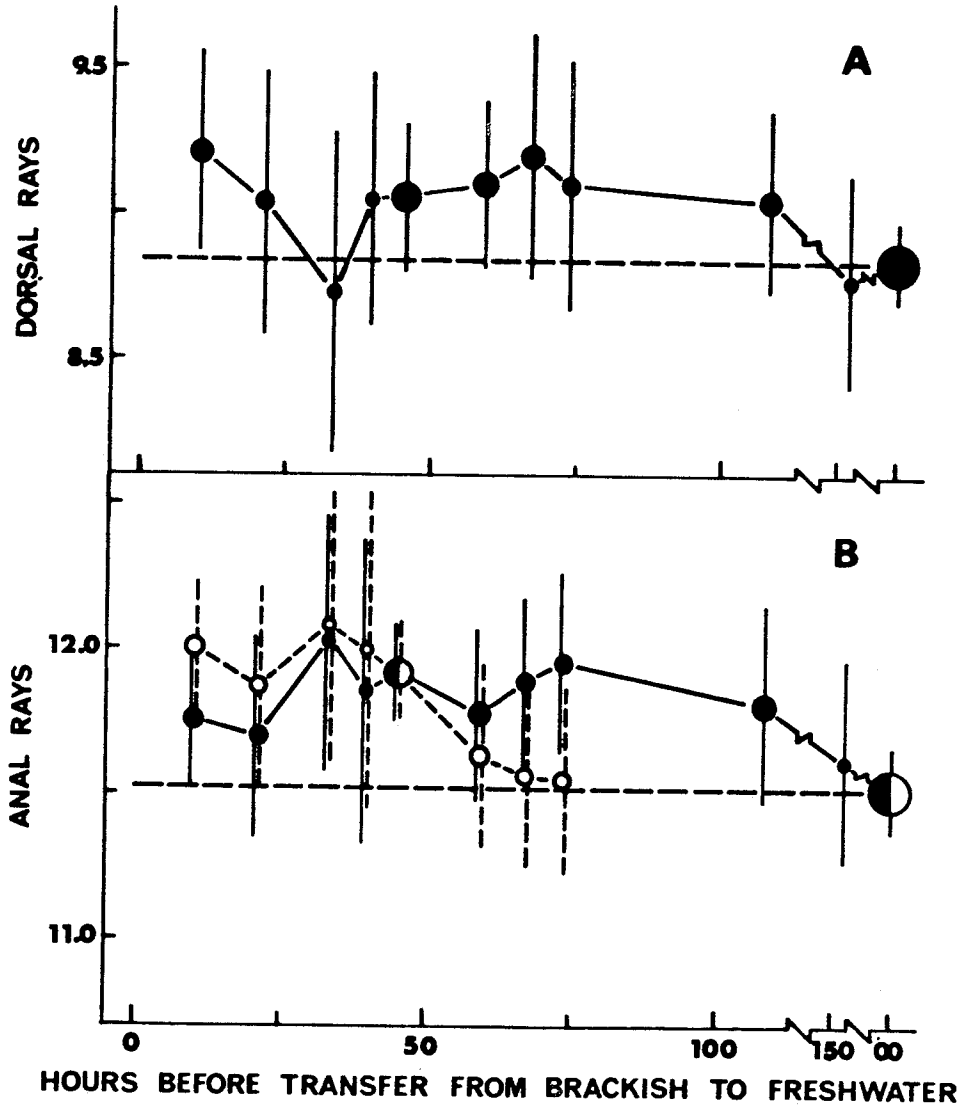

A
B
DORSAL RAYS
9.5
8.5
ANAL RAYS
12.0
11.0
0
50
100
150
00
HOURS BEFORE TRANSFER FROM BRACKISH TO FRESHWATER

Figure 10. Continued...

C. Caudal rays. Counts are unadjusted.

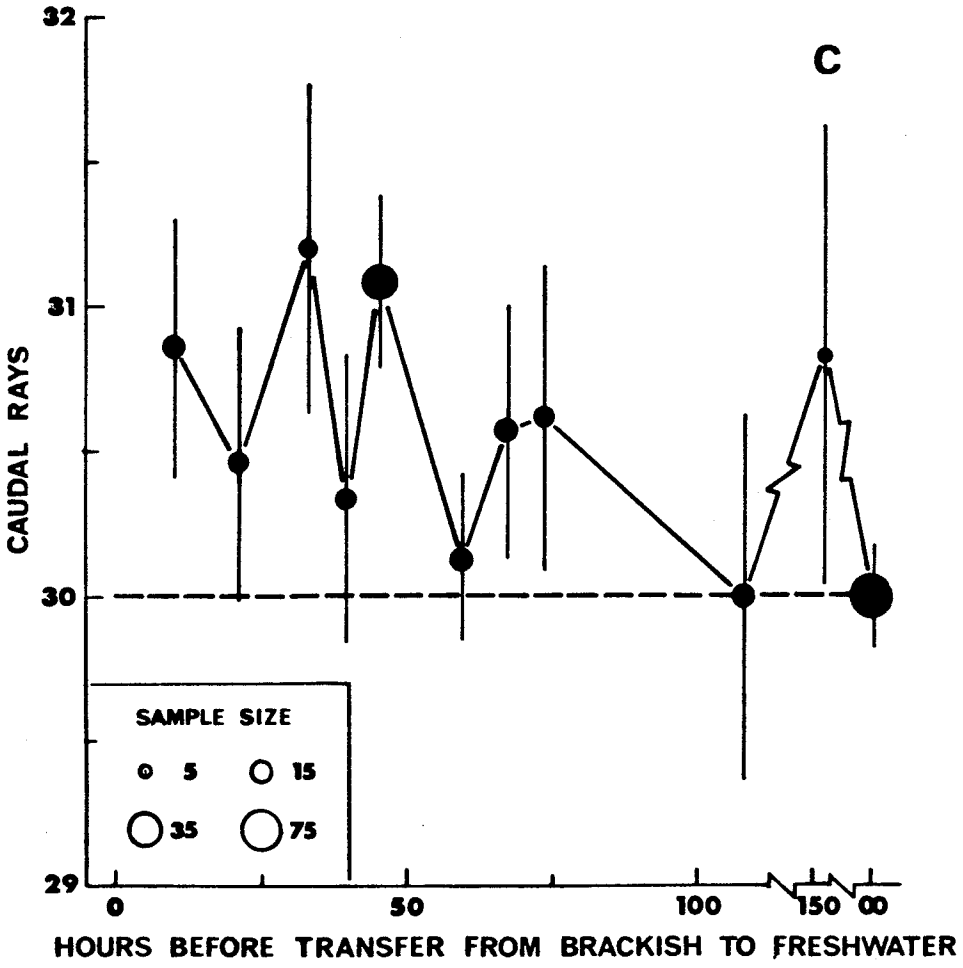

C
32
31
30
29
CAUDAL RAYS
SAMPLE SIZE
5
15
35
75
0
50
100
150
200
HOURS BEFORE TRANSFER FROM BRACKISH TO FRESHWATER

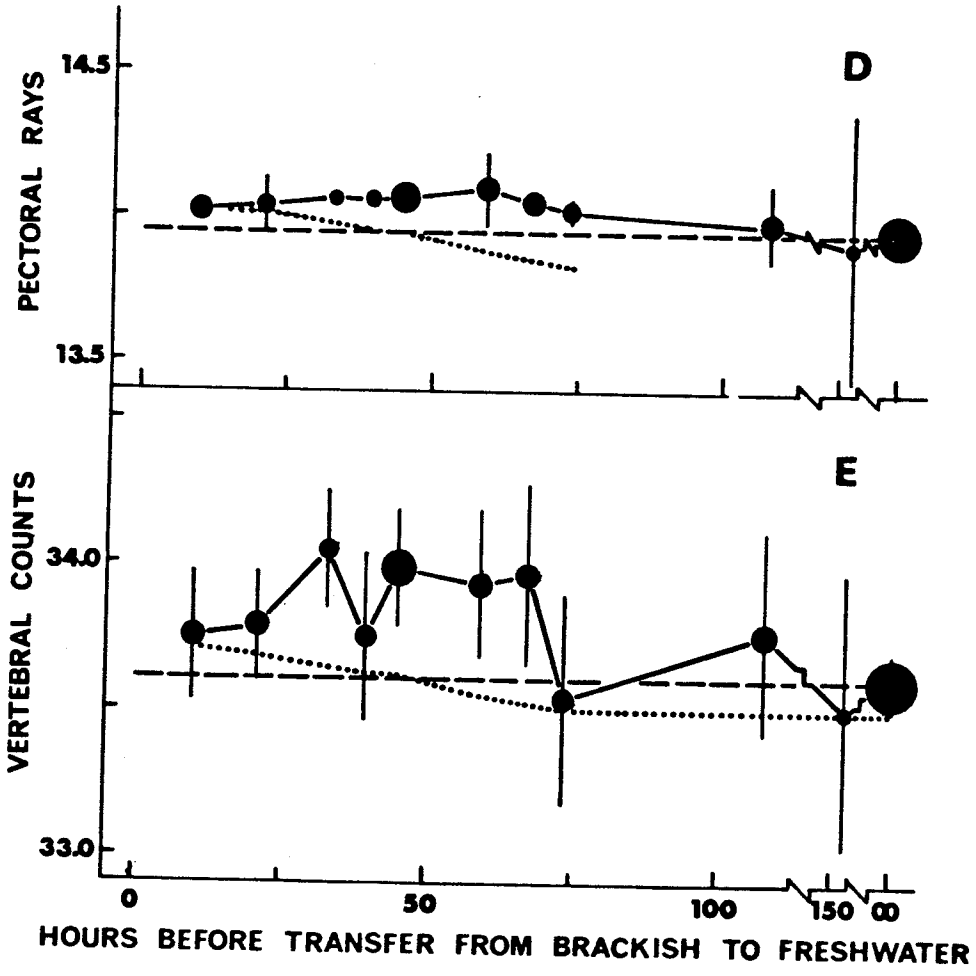
Figure 10 Continued...

D. Pectoral rays. Left and right counts are averaged on each fish and adjusted to the mean PRH of the brackish water control as follows:

$$Y_i(adj) = Y_i + 0.00080\ (84.2 - PRH_i)$$

Both an unadjusted value (broken line) and values adjusted to the mean intraparental incubation times in transfer lots (dotted line) are shown for the brackish water control.

E. Vertebral counts. Counts are unadjusted. Brackish water controls are shown as in panel D.


D
PECTORAL RAYS
14.5
13.5
E
VERTEBRAL COUNTS
34.0
33.0
0
50
100
150
∞
HOURS BEFORE TRANSFER FROM BRACKISH TO FRESHWATER

breaks varied among meristic series.

Transfer from brackish to freshwater at stages between 10 and 110 h of development appeared to increase dorsal ray counts relatively uniformly (Figure 10A). The low count in the small group with a mean transfer time of 33 h might be partly due to inaccurate adjustment for extreme confounding with PRH (5 of the 6 fish were laid less than 6 days after the onset of oviposition). The mean number of dorsal rays in the small group transferred after an average of 152 h of development (about the time of fixation of dorsal ray number - see Section 3.10) is similar to that of the brackish water control.

Transfer to freshwater after 10 - 45 h of development uniformly increased adjusted anal ray counts relative to the brackish water control (Figure 10B). Counts adjusted for variation in intraparental incubation time decreased to values near the brackish water control in lots transferred after about 50 - 75 h of development. Adjusted counts could not be predicted at longer intraparental incubation times, but an effect of salinity transfer after 75 h of development is expected since anal ray counts are not fixed until about 140 h after fertilization at 25C (Section 3.10). If no adjustment is made for varying duration of intraparental incubation, mean anal ray counts of all groups transferred to freshwater before about 110 h of development are similarly elevated above the brackish water control with only minor, irregular fluctuations.

Mean numbers of caudal rays in groups transferred to freshwater

before about 80 h of development fluctuated widely at values above the brackish water control (Figure 10C). No pattern of response is obvious, except that mean counts tend to decrease with increasing incubation time before salinity transfer.

Since left and right pectoral fins responded similarly to salinity effects (Appendix F), only average pectoral ray counts (averaged on each fish) are shown in Figure 10D. Pectoral ray counts unadjusted for varying intraparental incubation times showed little variation with respect to incubation salinity or stage at transfer between salinities. The mean counts of lots transferred to freshwater after 10 - 75 h of incubation displayed a small, relatively uniform increase above the brackish water control, although counts appeared to peak slightly at intermediate transfer times. Mean counts of fish transferred after averages of 108 and 152 h of development straddled the brackish water control. A more sharply dome-shaped response to salinity breaks is suggested using brackish water control values adjusted for variation in intraparental incubation time, but the validity of this adjustment is questionable (since the significance of regressions between intraparental incubation time and pectoral ray count may be an artifact of low variability and highly discrete data).

Transfer to freshwater at any time between 10 and 75 h after fertilization increased vertebral counts relative to the brackish water control (Figure 10E). Increases tended to be greatest in lots transferred at intermediate developmental stages (30 - 75 h after fertilization), particularly if adjustment is made for confounding

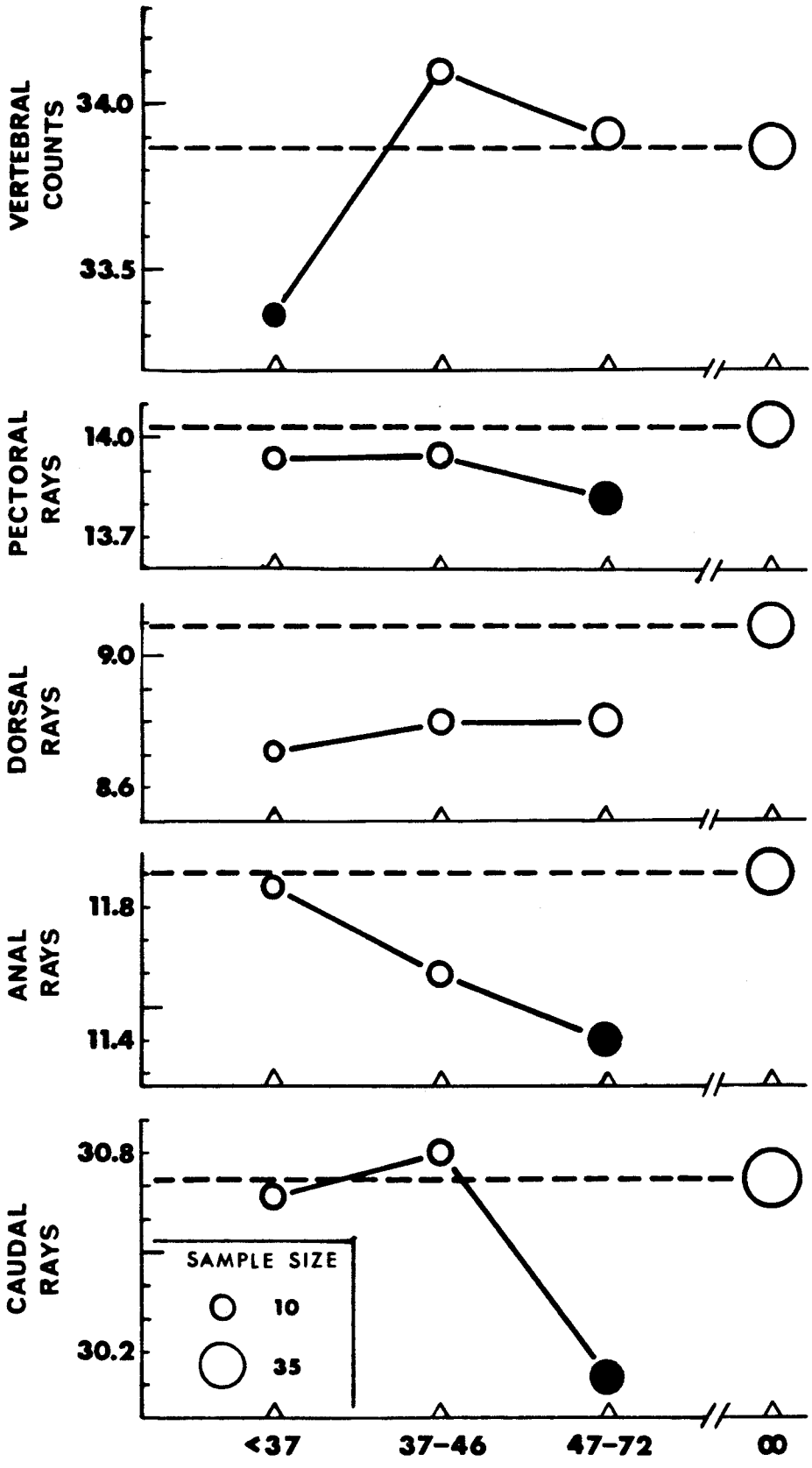
with intraparental incubation time. The pattern of response is reasonably consistent with a fixation time near 80 h after fertilization (the time suggested by temperature breaks (Section 3.10)). Only vertebral counts are shown in Figure 10, but total numbers of vertebral arches and centra responded similarly (Appendix F). Most vertebral variation associated with incubation salinity occurred in the caudal region (Appendix F).

The effect of sustained freshwater incubation on meristic counts cannot be determined from these data. Counts near or slightly greater than those produced by sustained brackish water incubation are probable, assuming patterns of response to salinity breaks similar to the types produced by tmperature breaks (see Section 4.5).

3.6 Incubation Light Intensity

Figure 11 summarizes meristic responses to tranfers to darkness during incubation. Fish transferred to darkness early in development had significantly fewer vertebrae than those incubated in light; transfer to darkness at intermediate stages tended to increase vertebral counts, but not significantly so; vertebral counts were unaffected by transfer to darkness late in development. Effects of transfers to darkness on total arch and centrum counts resembled the effects on vertebral counts, except that increases in centrum counts of fish transferred at intermediate stages approached significance

Figure 11. Meristic counts produced by transfers to darkness during development. Circle areas are proportional to sample sizes. Mean counts plotted as solid circles are significantly ($p < .05$) different from counts produced by sustained incubation in light (indicated by broken lines); those plotted as open circles are not significantly different from the latter.


VERTEBRAL COUNTS
34.0
33.5
PECTORAL RAYS
14.0
13.7
DORSAL RAYS
9.0
8.6
ANAL RAYS
11.8
11.4
CAUDAL RAYS
30.8
30.2
SAMPLE SIZE
10
35
<37
37–46
47–72
∞
HOURS BEFORE TRANSFER TO DARKNESS

(Appendix G). Transfer to darkness during incubation usually decreased numbers of pectoral, anal and caudal rays relative to the light control, but decreases were significant only among fish transferred relatively late in development. Transfers to darkness at all stages decreased dorsal ray counts, but not significantly so (except in a subsample of uniform GPTH (Appendix G)). Very small sample sizes may account for the lack of statistically significant effects in some cases. None of the singificant effects of transfers to darkness can be attributed to confounding with PRH, GPTH or intraparental incubation time (Appendix G).

These data demonstrate that light intensity experienced during incubation significantly influences numbers of vertebrae and most fin rays in *R. marmoratus,* but provide no definite indication of the relative effects of sustained incubation in light or darkness. Assuming patterns of response to light breaks similar to those observed to temperature breaks (Sections 3.10 and 4.6), these results suggest that sustained incubation in darkness might produce meristic counts similar to or lower than those produced by sustained incubation in light at an intensity of 80 - 85 ft.c.

3.7 Date of Collection

Mean meristic counts of fish collected between October 1976 and March 1977 did not differ significantly from those of fish collected between May and October 1977, comparing subsamples of the

25P25I-FW series with uniform GPTH and similar average PRH and intraparental incubation times (Appendix H). Apparently, no net systematic influences on meristic counts occurred over time in this study, despite differences in parents and incubation containers and slight variation in light intensity between the two main sampling periods.

3.8 Post-hatching Thermolability

Mean meristic counts of fish transferred to 30C within 1 - 2 days of hatching did not differ significantly from those of fish kept at 25C after hatching (Appendix I). Differences were not obscured by confounding with PRH, GPTH or intraparental incubation time in this comparison (Appendix I). Apparently, all meristic counts examined in this study are fixed by or shortly after hatching in *R. marmoratus*. However, the possibility that slight thermolability may have persisted in some meristic series after hatching is not entirely precluded by the results of this comparison because of the small number of fish transferred to 30C after hatching.

3.9 Parental Temperature History

To investigate the influence of parental temperature history on numbers of meristic parts, counts of fish conceived soon and long after their parents had been transferred from 25C to 30C were compared (Table 10). Estimated mean times between parental transfer

TABLE 10. Effect of time since parental temperature transfer from 25C to 30C on meristic counts of offspring reared at 30C. The most significant probabilities are underlined.

Meristic Series	Fish produced soon after parental temperature transfer (N=18)* Mean	S.D.	Fish produced long after parental temperature transfer (N=9)† Mean	S.D.	Significance
Abdominal arches	12.00	0.00	12.00	0.00	1.00
Caudal arches	21.78	0.43	21.56	0.53	0.25
Total arches	33.78	0.43	33.56	0.53	0.25
Abdominal centra	11.94	0.24	12.00	0.00	0.49
Caudal centra	21.28	0.46	20.89	0.33	<u>0.034</u>
Total centra	33.22	0.55	32.89	0.33	0.11
Total vertebral counts	33.53	0.36	33.22	0.36	<u>0.050</u>
Right pectoral rays	13.61	0.50	13.33	0.50	0.19
Left pectoral rays	13.67	0.48	13.22	0.44	<u>0.029</u>
Average pectoral rays	13.64	0.38	13.28	0.36	<u>0.025</u>
Dorsal rays	8.94	0.54	9.11	0.33	0.41
Anal rays	11.94	0.43	12.00	0.00	0.72
Caudal rays	30.56	0.98	29.44	0.73	<u>0.006</u>

* N = 17 for anal rays

† N = 7 for anal rays

and fertilization in the two groups were 5.5 and 102.4 days, respectively. Only one fish in the former group was conceived more than 10 days after parental temperature transfer; all those in the latter group were conceived more than 40 days after parental transfer. All fish in both groups were incubated in freshwater at 30C. All parents used in this experiment had been previously held at 25C throughout life.

Fish conceived soon after parental transfer to 30C had significantly greater vertebral and pectoral and caudal ray counts than did those conceived long after parental transfer. Differences in total numbers of vertebral arches and centra between the two groups resembled the difference in vertebral counts, but were not significant. Vertebral differences were due entirely to variation in the caudal region. No significant differences in numbers of anal or dorsal rays occurred between fish conceived soon and long after parental transfer to 30C.

The meristic differences between fish conceived soon and long after parental temperature transfer cannot be ascribed to confounding with extraneous variables. All fish were of known 25C grandparentage. Mean PRH was the same in the two groups (63.3 and 62.1 days, respectively). Average intraparental incubation time differed between the two groups (27.1 vs. 45.2 h, respectively), but cannot account for meristic differences of the magnitudes observed in this comparison. This is particularly true for pectoral ray counts: variation in mean

numbers of pectoral rays related to time between fertilization and parental temperature transfer was much greater than the maximum variation observed in response to any other factor except incubation temperature (Section 3.10).

Meristic differences related to time between fertilization and parental temperature transfer indicate either an effect on meristic counts of the actual temperature experienced by parents, or a "shock" effect associated with the transfer of parents between temperatures before fertilization of the eggs under discussion. Assuming the former, the above comparison indicates that:

1) a parental temperature of 30C produces fewer vertebrae and pectoral and caudal fin rays in offspring than does one of 25C;

2) for these meristic series, the maximum influence of a new parental temperature is not transmitted to offspring until parents have been exposed to the new temperature for at least about a week (and possibly much longer); and,

3) parental temperature history either does not influence numbers of dorsal and anal fin rays in offspring, or the influence of a new parental temperature on these counts is already maximal in offspring conceived within fewer than 5 days of parental transfer.

Further information about the influence of parental temperature history on meristic counts is provided by temperature break experiments described below.

3.10 Temperature Break Experiments

Figures 12 - 16 show the results of reciprocal temperature break experiments between 25C and 30C. To facilitate comparisons between the two experiments, the time scale is expanded for the 30C to 25C break experiment so that approximately the same scale of developmental stages is used for both experiments (but this is not meant to imply precise equivalence between all developmental processes at any "developmental stage" at different temperatures). Mean counts of transfer lots are plotted in the figures at the estimated mean incubation times before transfer of all fish within each lot. Due to the long duration of late developmental stages, the possible range of actual incubation times before transfer in any one lot is great in those transferred after about 80 h of development at 25C. The curves shown in these figures are computer simulations produced by fitting the meristic response model of C.C. Lindsey and A.N. Arnason (Arnason *et al.* 1978) to the data in this study. All computer fitting and simulations were kindly performed by C.C. Lindsey. The meristic response model and its fit to these data will be discussed below (Section 4.7).

These experiments also provide information about the effects of parental temperature on meristic counts. Fish transferred from 25C to 30C during development were produced by parents held at 25C; those transferred from 30C to 25C were produced by parents which had been held at 30C for more than 40 days. Control counts for sustained

incubation at 25C of eggs from parents held at 25C were provided by the 25P25I-FW series. For meristic series significantly affected by parental temperature, control counts for sustained incubation at 30C of eggs from parents held at 30C were provided by fish conceived long after parental temperature transfer in the 30P30I series. For these meristic series, the approximate values of the 30C controls for fish with parents held at 25C were surmised from the direction of the parental temperature effect (Section 3.9) and from trends evident in meristic responses to early temperature breaks. The entire 30P30I series provided sustained 30C control counts of meristic series unaffected by parental temperature history.

3.10.1 Extraneous Sources of Variation

Parental reproductive history varied among the four treatment series in these experiments. Parents with long reproductive histories (PRH > 30 days) produced 71, 83 and 96% of the fish in the 25P30I, 30P30I and 30P25I series, respectively, but only 38% of the fish in the 25P25I-FW series. In the latter series, 38% of the fish were produced from eggs laid within 5 days of the onset of oviposition. To eliminate most confounding with PRH, fish with PRH of 5 days or less are excluded from analyses of all meristic series except caudal fin rays (which apparently are not affected by PRH). This subsampling altered the mean numbers of dorsal and anal rays in the 25P25I-FW series slightly, but had little effect on any other mean meristic counts except those of some late transfer lots in the 25P30I treatment

(which contained a high proportion of fish laid soon after the onset of oviposition). About 15% of the fish in the 25P30I treatment were of unknown PRH. These fish are included in analyses since their exclusion did not alter mean meristic counts. Details are given in Appendix J.

Most fish in all treatments were of known 25C grandparentage (100, 100, 77 and 66% in the 30P25I, 30P30I, 25P30I and 25P25I-FW series, respectively). Most of the remaining fish were of unknown GPTH. Five and 3%, respectively, of the 25P30I and 25P25I-FW series were of known 30C GPTH. No differences attributable to GPTH occurred between the entire sample and the subsample of known 25C grandparentage in any meristic series in any treatment (Appendix J). Accordingly, fish of all GPTH are included in Figures 12 - 16.

Mean incubation times before both oviposition and transfer to freshwater were similar among the four treatments. However, within the two temperature break series, mean intraparental incubation time generally increased with increasing developmental time before temperature transfer (Appendix J). Transfer from brackish to freshwater generally coincided approximately with temperature transfers. For most meristic series having significant regressions with duration of intraparental incubation in the freshwater series, values of the 25C control adjusted to the mean intraparental incubation times in transfer lots are shown for each lot in the 25C to 30C break experiment (Figures 12 and 13). This procedure displays the magnitude of

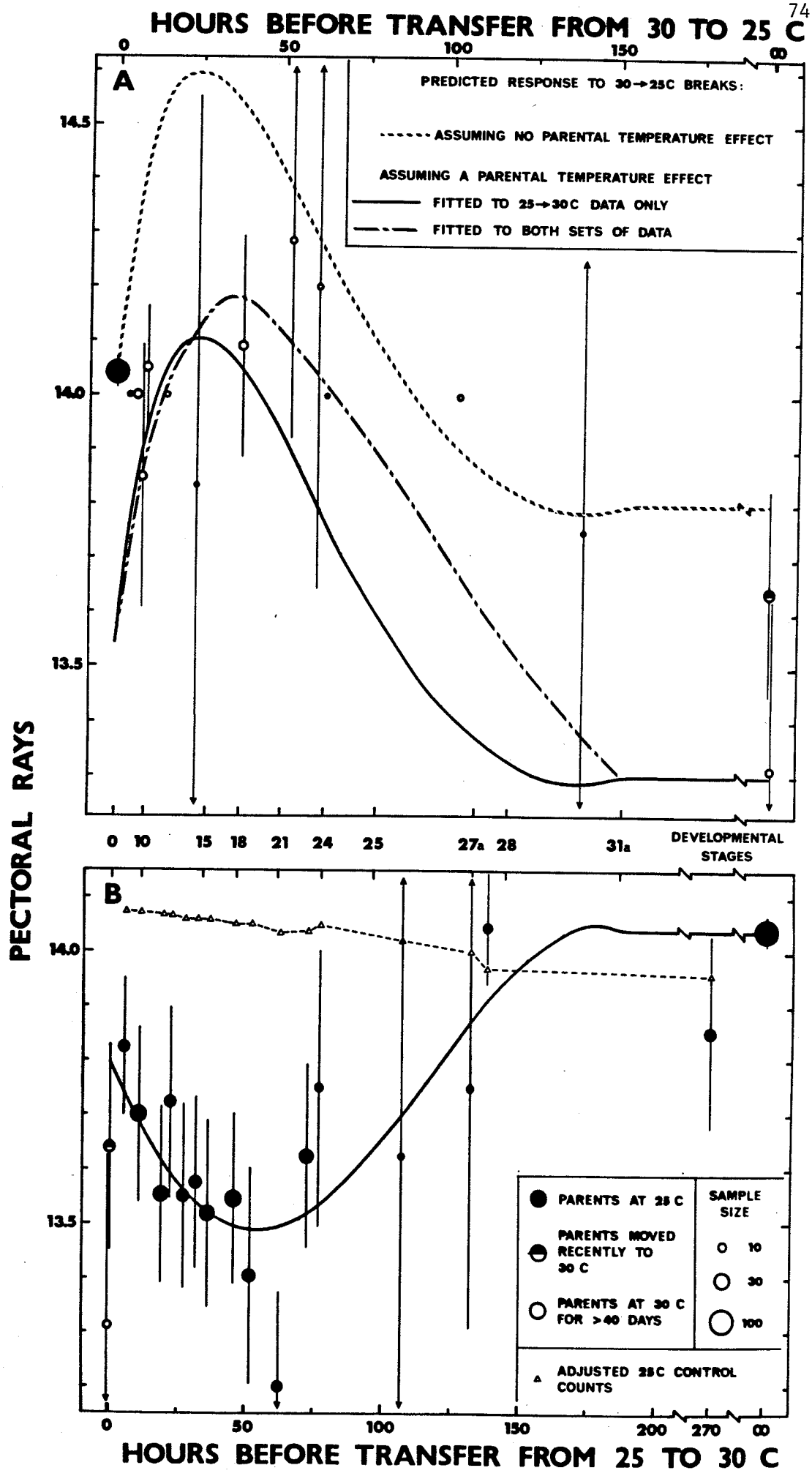
variation attributable to transfers to the extraparental environment and freshwater in these experiments, but relationships are probably not actually linear. Since the net effect of transfers to freshwater and the extraparental environment is in fact relatively constant for most meristic series up to the time of meristic fixation (Figure 10), the true patterns of meristic response to temperature breaks should not be materially obscured by confounding with times of oviposition and salinity breaks. Caudal fin ray counts are an exception due to their relatively great response to transfers to freshwater during development and are accordingly adjusted for variation in the times of transfer between salinities (see below).

3.10.2 Pectoral Rays

Figure 12 shows responses of pectoral fin ray number to temperature breaks between 25C and 30C. Since left and right pectoral fins responded similarly, only average pectoral ray counts are shown. Responses to parental temperature transfers, and temperature breaks early during development, suggested a 30C control value of about 13.8 - 13.9 pectoral rays for offspring of parents held at 25C.

The 25C to 30C breaks indicated that pectoral ray count is labile to temperature influence from about fertilization to at least about stage 28 (about 144 h of development at 25C). Transfers from 25C to 30C during a prolonged period from about fertilization to stage 26 produced extralimitary counts lying in the direction anticipated from

Figure 12. Pectoral ray counts produced by different parental temperature histories and by temperature breaks between 25C and 30C at indicated developmental stages. Circle areas are proportional to sample sizes. Vertical lines show 95% confidence intervals. Triangles show 25C control counts adjusted to the mean intraparental incubation times in transfer lots (using the regression equation for the FW series in Table 5). Curves show fits of these data or responses predicted by the meristic response model (see text).


HOURS BEFORE TRANSFER FROM 30 TO 25 C
0
50
100
150
∞
A
PREDICTED RESPONSE TO 30→25C BREAKS:
ASSUMING NO PARENTAL TEMPERATURE EFFECT
ASSUMING A PARENTAL TEMPERATURE EFFECT
FITTED TO 25→30C DATA ONLY
FITTED TO BOTH SETS OF DATA
14.5
14.0
13.5
PECTORAL RAYS
0 10 15 18 21 24 25 27a 28 31a
DEVELOPMENTAL STAGES
B
14.0
13.5
PARENTS AT 25 C
PARENTS MOVED RECENTLY TO 30 C
PARENTS AT 30 C FOR >40 DAYS
SAMPLE SIZE
10
30
100
ADJUSTED 25C CONTROL COUNTS
0
50
100
150
200
270
∞
HOURS BEFORE TRANSFER FROM 25 TO 30 C

the effect of sustained incubation at 30C. This type of extra-limitary response is termed "overcompensation" (Ali and Lindsey 1974). Overcompensation was greatest among fish transferred at about stages 19 - 21.

The curve shown in Figure 12B was produced by fitting the meristic response model of Arnason *et al.* (1978) to the 25C to 30C transfer data, assuming a 30C control of 13.8 rays and fixation at 187 h of development at 25C. This late fixation time provided the best fit to the data, but simulations assuming earlier fixation also fitted reasonably well (C.C. Lindsey, pers. comm.). The absence of lots transferred between 144 and 270 h after fertilization allows the possibility of thermolability and a slight paradoxical reaction to temperature breaks after 144 h of development.

The predicted net effect of transfers to freshwater and to the extraparental environment are negligible compared with responses to temperature breaks during the labile period (Figure 12B). The low mean pectoral ray count of fish transferred to 30C 270 h after fertilization may however be partly due to "sustained" incubation in brackish water. These fish were transferred to freshwater after the fixation of pectoral ray counts.

The 25C to 30C break experiment supports the conclusion that parental temperature affects numbers of pectoral rays. The results of this experiment conform to expected patterns of response to temperature breaks (see Section 4.6) only if fish from parents at 25C are assumed to have more pectoral rays than those from parents

held at 30C. Fish (with parents at 25C) transferred from 25C to 30C very early in development (at stages 2 - 8b) have significantly more pectoral rays than those incubated at a sustained temperature of 30C but conceived soon ($p < .033$) or long ($p < .001$) after parental transfer to 30C. The effect of early transfer from 25C to 30C is apparently in the direction which obscures differences due to different parental temperatures in this comparison. Thus, this comparison indicates a significant effect of new parental temperature on pectoral ray counts of offspring conceived within 5 days of parental transfer to the new temperature.

The response of pectoral ray counts to breaks from 30C to 25C during development conform to the expectation that reciprocal temperature break experiments should elicit meristic responses that are roughly mirror images (see Ali and Lindsey 1974), but again only if an effect of parental temperature is assumed (Figure 12A). If no effect of parental temperature were assumed, fish transferred from 30C to 25C early in development would appear to have the same number of pectoral rays as those reared at a sustained temperature of 25C; that is, no early thermolability or extralimitary responses to temperature breaks would be evident. If on the other hand a parental temperature effect of the magnitude indicated by the 25C to 30C breaks (about 0.5 rays) is assumed, the responses to breaks from 30C to 25C are closely comparable to those produced by the reciprocal experiment: pectoral ray number appears to be thermolabile very early in development and responds to early temperature breaks with

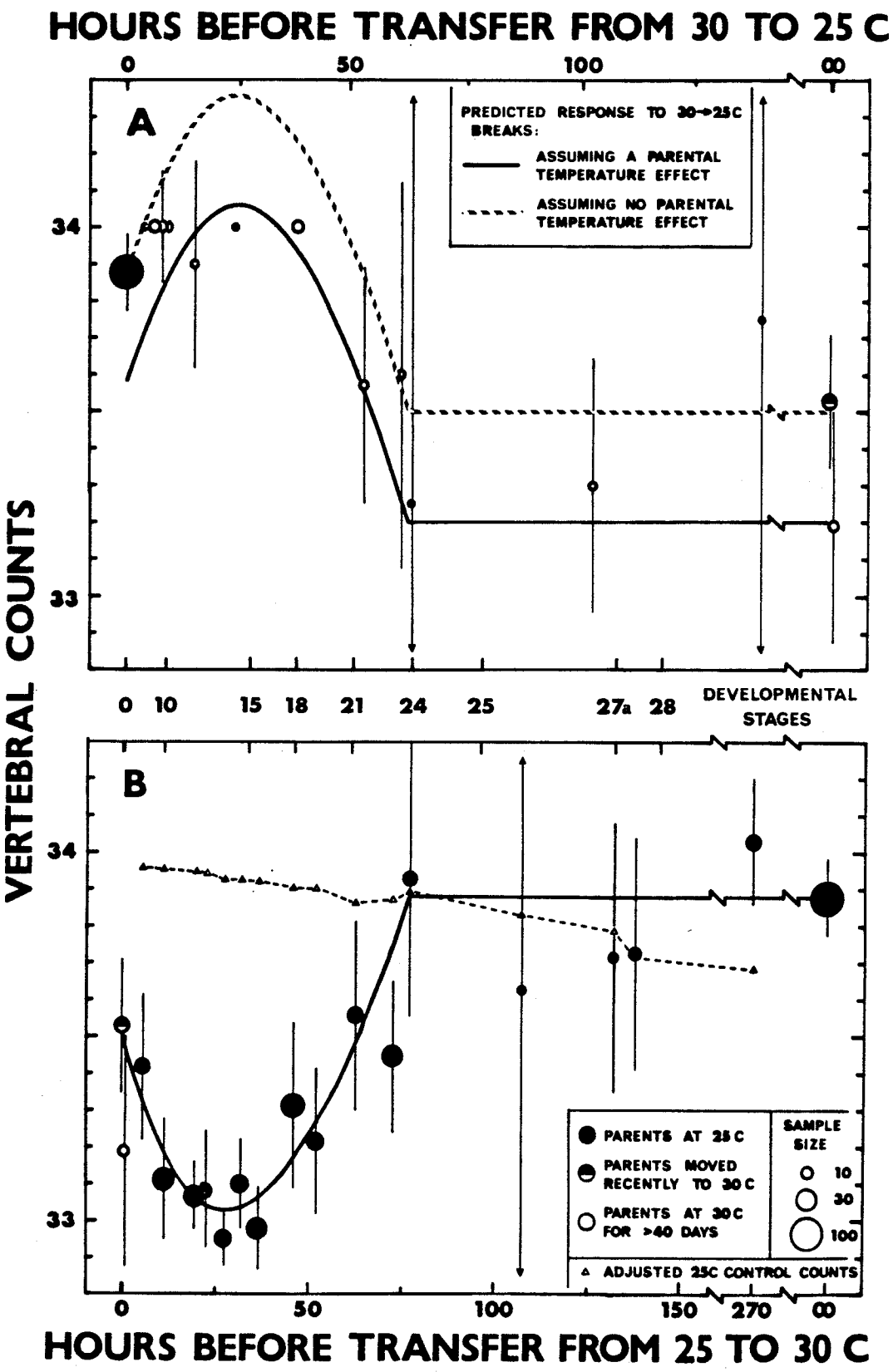
overcompensation. The response to breaks from 30C to 25C predicted by the meristic response model (using parameter values obtained by "optimizing" on the 25C to 30C break data) fit the data well assuming an effect of parental temperature, but poorly assuming none. A slightly improved fit is obtained by optimization on both sets of data, assuming a parental temperature effect of 0.5 rays (C.C. Lindsey, pers. comm.; Figure 12A).

3.10.3 Vertebrae

The response of vertebral counts to temperature breaks during development resembled that of pectoral ray number (Figure 13). Parental temperature transfers and early temperature breaks from 25C to 30C suggested a 30C control of 33.5 or more vertebrae for fish from parents at 25C. Breaks from 25C to 30C indicated vertebral thermolability from about fertilization to about stage 25 (about 80 h of development at 25C). Early temperature breaks produced striking overcompensation.

The curve shown in Figure 13B was produced by fitting the meristic response model to the 25C to 30C break data, assuming a 30C control of 33.5 vertebrae and a time to fixation of 77 h at 25C. The use of 33.5 vertebrae as the 30C control was conservative; a higher value is possible if overcompensation is considerable even in the earliest transfer lot. A higher value would indicate an even greater effect of long-term parental temperature on vertebral counts and an effect of a new parental temperature on vertebral counts of offspring

Figure 13. Vertebral counts produced by different parental temperature histories and by temperature breaks between 25C and 30C at indicated developmental stages. Circle areas are proportional to sample sizes. Vertical lines show 95% confidence intervals. Triangles show 25C control counts adjusted to the mean intraparental incubation times in transfer lots (using the regression equation for the FW series in Table 4). Curves show the fit of these data or responses predicted by the meristic response model (see text).


HOURS BEFORE TRANSFER FROM 30 TO 25 C
0
50
100
∞
A
PREDICTED RESPONSE TO 30→25C BREAKS:
ASSUMING A PARENTAL TEMPERATURE EFFECT
ASSUMING NO PARENTAL TEMPERATURE EFFECT
34
33
VERTEBRAL COUNTS
0 10 15 18 21 24 25 27a 28
DEVELOPMENTAL STAGES
B
34
33
PARENTS AT 25 C
PARENTS MOVED RECENTLY TO 30 C
PARENTS AT 30C FOR >40 DAYS
SAMPLE SIZE
10
30
100
ADJUSTED 25C CONTROL COUNTS
0
50
100
150
270
∞
HOURS BEFORE TRANSFER FROM 25 TO 30 C

conceived within 5 days of parental temperature transfer (as observed for pectoral ray counts).

The predicted net effect of transfers to freshwater and to the extraparental environment are negligible compared with vertebral responses to temperature breaks (Figure 13B). The relatively low mean vertebral counts of some lots of fish transferred between temperatures after the fixation of vertebral number might be partly due to sustained incubation in brackish water during the period of vertebral lability.

As in the case of pectoral rays, vertebral responses to breaks from 25C to 30C conform to expected patterns (see Section 4.6) only if an effect of parental temperature history is assumed. However, the mean vertebral count of fish in the earliest transfer lot did not differ significantly from those of fish reared continuously at 30C and conceived soon or long after parental transfer to 30C ($p > 0.6$ and $p > 0.2$, respectively). Strong overcompensation in the earliest transfer lot probably obscured vertebral differences due to parental temperatures in these comparisons. The earlier developmental time at transfer to freshwater among fish experiencing early temperature breaks might also have obscured parental temperature effects slightly.

Vertebral responses to breaks from 30C to 25C reflect responses to the reciprocal breaks, showing early thermolability and overcompensation, if an effect of parental temperature is assumed

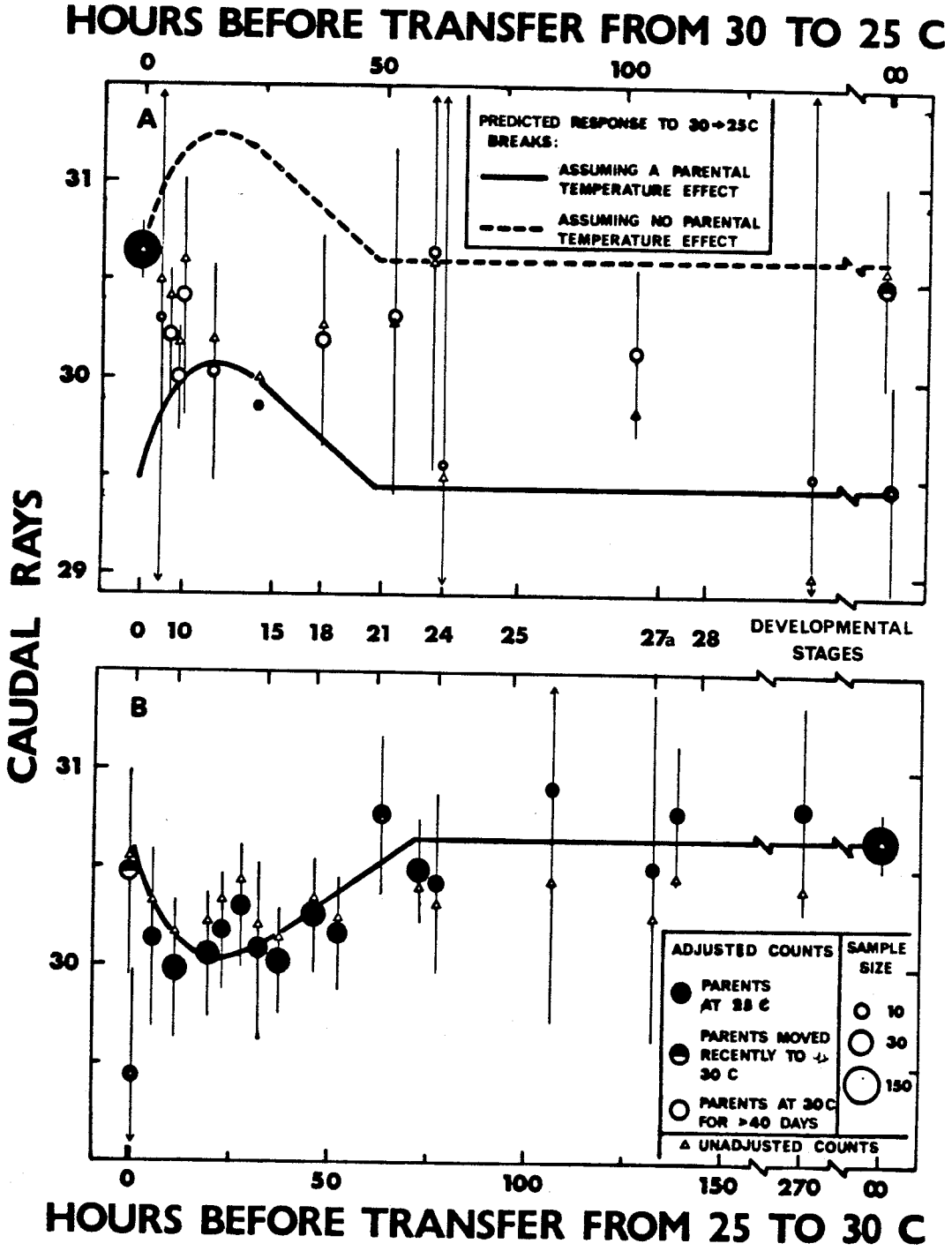
(Figure 13A). The response to breaks from 30C to 25 C predicted by the meristic response model (using paramter values estimated by optimization on the 25C to 30C break data) fit the data slightly better assuming a parental temperature effect. Selection of a value greater than 33.5 vertebrae for the 30C control with parents at 25C would probably further improve this fit assuming an effect of parental temperature and worsen it assuming none.

Only total vertebral counts were reported for brevity. Total arch and centrum counts displayed similar trends, although centrum counts responded less markedly and regularly to temperature breaks (indicating the importance of complex vertebrae in these responses). Most vertebral variation related to temperature breaks resulted from alterations in the number of caudal vertebrae. Details are given in Appendix J.

3.10.4 Caudal Fin Rays

Temperature break experiments did not elicit as clear a pattern of response in caudal fin ray counts as in vertebral and pectoral ray counts (Figure 14). Parental temperature transfers suggested a 30C control of about 30.6 or more caudal rays for fish from parents at 25C. Since they produced no clear trend in caudal ray repsonses, early temperature breaks were unilluminating with regard to the 30C control value. Breaks from 25C to 30C appeared to depress caudal ray counts uniformly below the 25C control value, irrespective of transfer time (observe the triangles in Figure 14B). However, caudal ray responses

Figure 14. Caudal ray counts produced by different parental temperature histories and by temperature breaks between 25C and 30C at indicated developmental stages. Counts are adjusted to the mean intraparental incubation time of the 25C control (see text). Triangles show unadjusted counts. Circle areas are proportional to sample sizes. Verticle lines show 95% confidence intervals. Curves show the fit of these data or responses predicted by the meristic response model (see text).


HOURS BEFORE TRANSFER FROM 30 TO 25 C
0
50
100
∞
A
PREDICTED RESPONSE TO 30→25C BREAKS:
ASSUMING A PARENTAL TEMPERATURE EFFECT
ASSUMING NO PARENTAL TEMPERATURE EFFECT
31
30
29
CAUDAL RAYS
0 10 15 18 21 24 25 27a 28
DEVELOPMENTAL STAGES
B
31
30
ADJUSTED COUNTS
PARENTS AT 25 C
PARENTS MOVED RECENTLY TO 30 C
PARENTS AT 30C FOR >40 DAYS
SAMPLE SIZE
10
30
150
UNADJUSTED COUNTS
0
50
100
150
270
∞
HOURS BEFORE TRANSFER FROM 25 TO 30 C

to temperature breaks were probably obscured significantly by effects of transfer to freshwater. After adjustment to the mean intraparental incubation time of the 25C control[1], the caudal ray counts of fish experiencing early breaks from 25C to 30C were strikingly lower than the 25C control, while those of fish experiencing late temperature breaks were similar to the control value (Figure 14B). Assuming an effect of parental temperature, a pattern of response similar to those of vertebrae and pectoral rays is suggested; caudal ray count appears to be thermolabile from fertilization to about 72 h thereafter and responds to early temperature breaks with over-compensation.

The curve shown in Figure 14B is the response predicted by the meristic response model after fitting to the adjusted 25C to 30C break data, assuming a 30C control of 30.6 rays and a fixation time of 72 h of development at 25C. Again, a slightly higher 30C control value is possible. Simulations using a 30C control of 30.7 rays produced similar results, although the extralimitary response is defined as a "paradoxical reaction" in the latter case since the control count now lies higher at 30C than at 25C. (Extralimitary

---

[1]This adjustment was performed using the significant regression between caudal ray counts and duration of intraparental incubation in the 25P25I-FW series. This regression is apparently entirely due to the effects of transfers to freshwater during development. However, the actual response to such transfers is most probably not linear (although no other pattern of response was apparent in the analysis in Section 3.5) and may vary somewhat among different incubation temperatures. Thus, the adjustments are obviously only very approximate, but at least partly compensate for the relatively large increase in caudal ray counts among fish transferred to freshwater during the labile period relative to those of fish transferred after meristic fixation.

responses lying in the direction opposite to that predicted from the effect of sustained incubation at the temperature to which the transfer occurred are termed "paradoxical reaction" (Orska 1956, Ali and Lindsey 1974).)

The response of caudal ray counts to breaks from 30C to 25C mirrors the response to the reciprocal breaks, if an effect of parental temperature is assumed (Figure 14A). Responses predicted by the meristic response model (optimized on the 25C to 30C data) again fit the observed responses considerably better assuming a parental temperature effect than assuming none.

3.10.5 Dorsal and Anal Fin Rays

Figures 15 and 16 show the effects of temperature breaks on numbers of anal and dorsal fin rays, respectively. Parental temperature transfers failed to indicate any effect of parental temperature on these meristic series. Results of the break experiments conform to expected patterns of response without assuming a parental temperature effect. Accordingly, the entire 30P30I series was used to provide 30C controls for dorsal and anal ray counts in both break experiments.

Both meristic series appeared to be thermolabile from soon after fertilization until at least 120 h thereafter at 25C. The meristic response model fits the data reasonably well assuming no parental temperature effect and fixation times of 144 and 163 h

Figure 15. Anal ray counts produced by different parental temperature histories and by temperature breaks between 25C and 30C at indicated developmental stages. Circle areas are proportional to sample sizes. Vertical lines show 95% confidence intervals. Curves show fits of these data by the meristic response model.

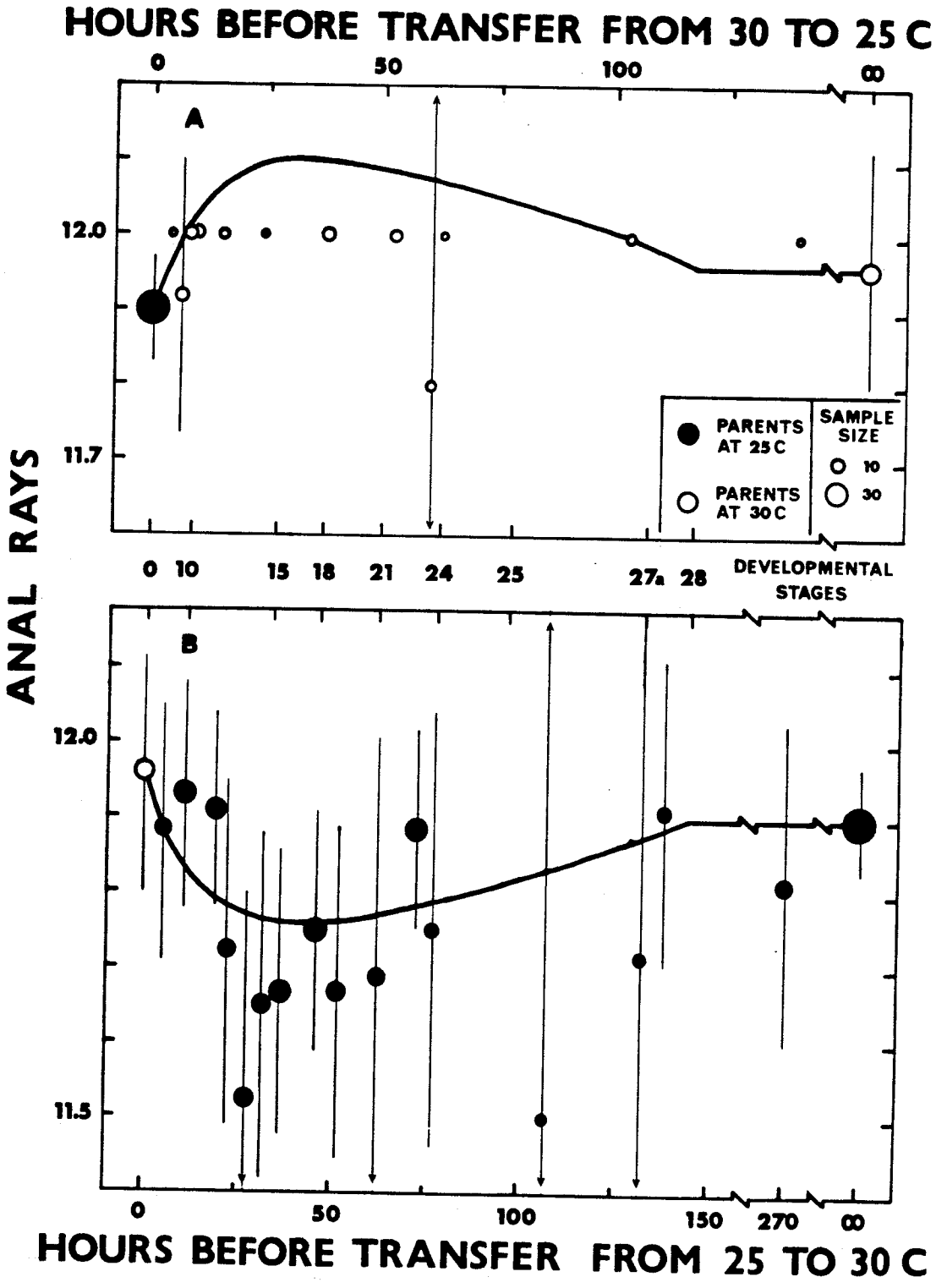
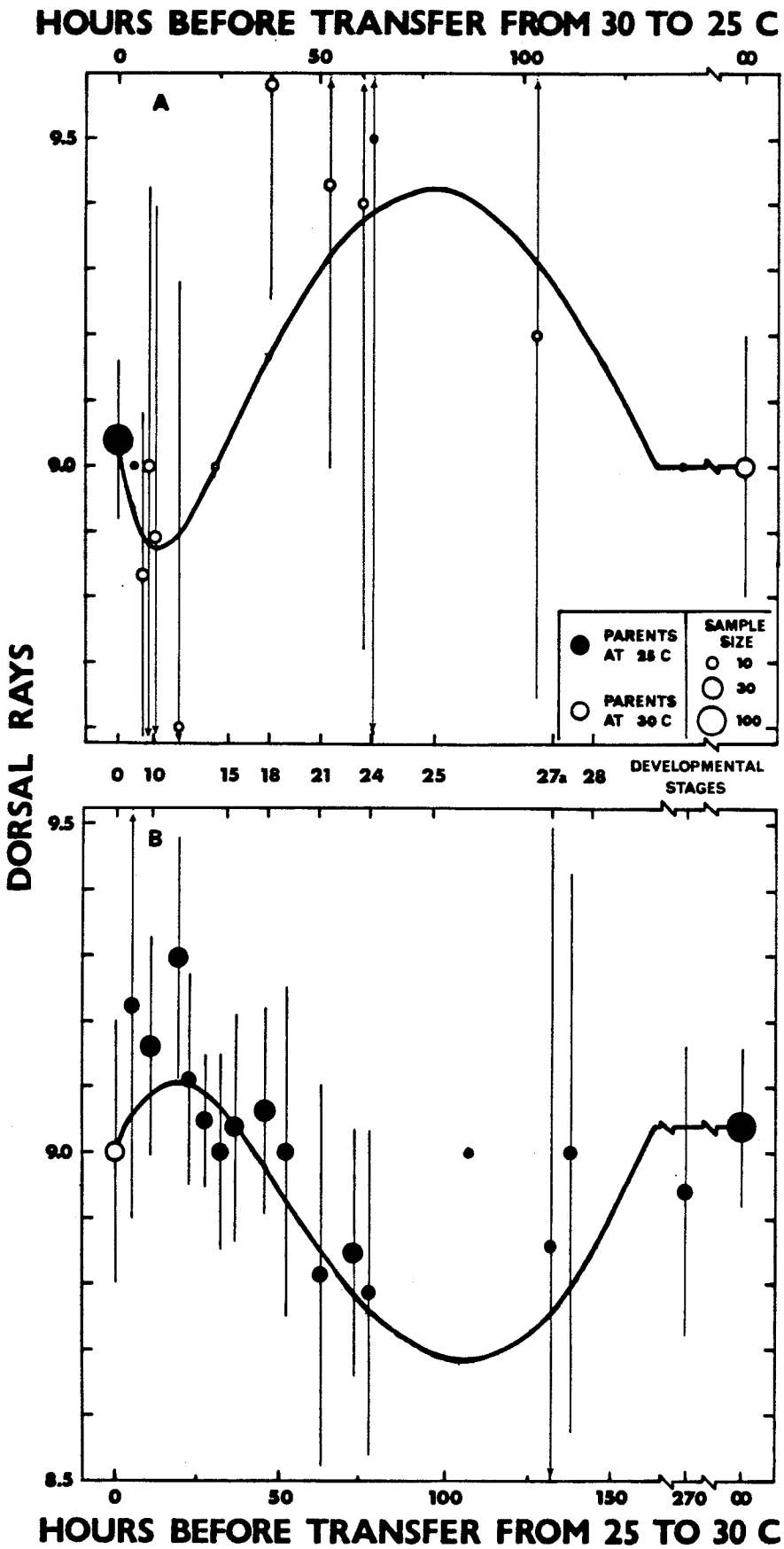

HOURS BEFORE TRANSFER FROM 30 TO 25 C
0
50
100
∞
A
12.0
11.7
PARENTS AT 25 C
PARENTS AT 30 C
SAMPLE SIZE
10
30
0 10 15 18 21 24 25 27a 28
DEVELOPMENTAL STAGES
ANAL RAYS
B
12.0
11.5
0
50
100
150
270
∞
HOURS BEFORE TRANSFER FROM 25 TO 30 C

Figure 16. Dorsal ray counts produced by different parental temperature histories and by temperature breaks between 25C and 30C at indicated developmental stages. Circle areas are proportional to sample sizes. Vertical lines show 95% confidence intervals. Curves show fits of these data by the meristic response model.


HOURS BEFORE TRANSFER FROM 30 TO 25 C
0
50
100
∞
A
9.5
9.0
PARENTS AT 25 C
PARENTS AT 30 C
SAMPLE SIZE
10
30
100
0
10
15
18
21
24
25
27a
28
DEVELOPMENTAL STAGES
DORSAL RAYS
B
9.5
9.0
8.5
0
50
100
150
270
∞
HOURS BEFORE TRANSFER FROM 25 TO 30 C

after fertilization at 25C for anal and dorsal rays, respectively. However, erratic fluctuation among mean counts of transferred lots was considerable, and fits of the model were poorer than those to vertebral and pectoral ray responses. Responses to reciprocal break experiments roughly mirrored each other in both meristic series. Temperature breaks at any time during the thermolabile period produced paradoxical reaction in anal ray number. In dorsal ray numbers early temperature breaks produced paradoxical reaction and later breaks caused overcompensation.

Patterns of response to temperature breaks were probably not modified by transfers to the extraparental environment and to freshwater, since the net effect of these transfers appeared to be relatively constant for dorsal and anal fin rays, at least during meristic labile periods (Figure 10). However, the relatively low counts among fish transferred between temperatures after meristic fixation might be partly attributable to these factors.

3.10.6 Summary

All five meristic series examined appeared to be thermolabile from fertilization to from 60 to 190 h thereafter at 25C. All were apparently fixed before hatching. Patterns of response to reciprocal break experiments were roughly mirror images for all five meristic series (assuming a parental temperature effect on numbers of vertebrae and pectoral and caudal rays). Early temperature breaks produced

striking overcompensation in vertebral and pectoral and caudal ray counts. Temperature breaks during the thermolabile period produced paradoxical reaction in anal ray counts. Early breaks elicited paradoxical reaction in dorsal ray counts; later breaks produced overcompensation.

Vertebral and pectoral and caudal ray responses to temperature breaks corroborated an effect of parental holding temperature on the numbers of these parts. Patterns of response resembled expected patterns only if offspring from parents held at 30C were assumed to have fewer vertebrae and pectoral and caudal fin rays than those from parents at 25C. These results indicate that the effect of parental temperature history reported in Section 3.9 is due to the actual temperature experienced by parents, rather than to a shock effect related to parental temperature transfer. Responses to temperature breaks were consistent with a lack of effect of parental temperature on numbers of anal and dorsal rays.

4. DISCUSSION

4.1 Vertebral Irregularities and Counting Methods

High frequencies of vertebral irregularities (notably fused centra and accessory arches) are reported in many meristic studies (for example, Gabriel 1944, Tåning 1944, Molander and Molander-Swedmark 1957). The treatment of these irregularities is accordingly an important (and often controversial) issue in studies of meristic variation.

Kandler (1932, 1935) and Tåning (1944) distinguished two main types of vertebral irregularities: "true fusions", defined by double neural and haemal arches, at least a trace of a suture in the centrum, and location anywhere in the vertebral column except the first and last vertebrae; and "complex vertebrae", characterized by accessory neural and/or haemal arches, usually an abnormally long centrum, and invariable location at the extreme anterior or posterior end of the vertebral column. Tåning (1944) further distinguished between complex post-cranial and pre-urostyle vertebrae, considering them to be the products of fundamentally different processes. In all studies making these distinctions, complex pre-urostyle vertebrae comprised the majority of vertebral irregularities, and fusions were usually rare (Gabriel 1944, Tåning 1944, Molander and Molander-Swedmark 1957, Canagaratnam 1959). Samples in the present study displayed similarly high frequencies of complex pre-urostyle vertebrae, but also relatively high proportions of complex post-cranial vertebrae and fusions

compared to the frequencies reported in other studies. The high frequency of fusions in *R. marmoratus* may be caused by oviposition during sensitive developmental stages, a trauma not experienced in the other species studied which displayed few fusions.

Most workers agree that vertebral arches are the most reliable indicators of the number of segments, at least in cases of "true fusions" (see Gabriel 1944, Molander and Molander-Swedmark 1957). The latter probably represent developmental abnormalities, involving secondary fusion of already determined segments through a disruption of influences regulating the chondrification of centra (see Section 4.4). Alternatively, these structures could be produced by secondary proliferations of arches from single elements. The centrum and arch counts of fish with highly irregular vertebral columns suggest however that the latter alternative is usually not involved. For example, in samples of medaka *Oryzias latipes* containing high frequencies of environmentally or genetically induced vertebral "fusions", mean numbers of centra are reduced drastically, but mean numbers of neural and haemal processes do not differ from those of normal fish (Yamamoto *et al.* 1963, Ogawa 1965). Thus, assignment of values equal to the numbers of pairs of arches seems appropriate for fused vertebrae.

The value that should be assigned to complex pre-urostyle vertebrae poses a more difficult problem. This problem might be of practical importance because the frequency of complex pre-urostyle vertebrae may vary among experimental treatments (Tåning 1944,

Molander and Molander-Swedmark 1957, this study). The appropriate value depends upon which of the following these structures represent:

1) a secondary fusion of two segments (count = 2);
2) proliferation from a single element (count = 1); or,
3) incipient segments which did not "succeed in separating completely" during the initial determination of segments (Gabriel 1944) or attempts to realize "fractional" vertebral counts (Tåning 1944), (count = 1.5).

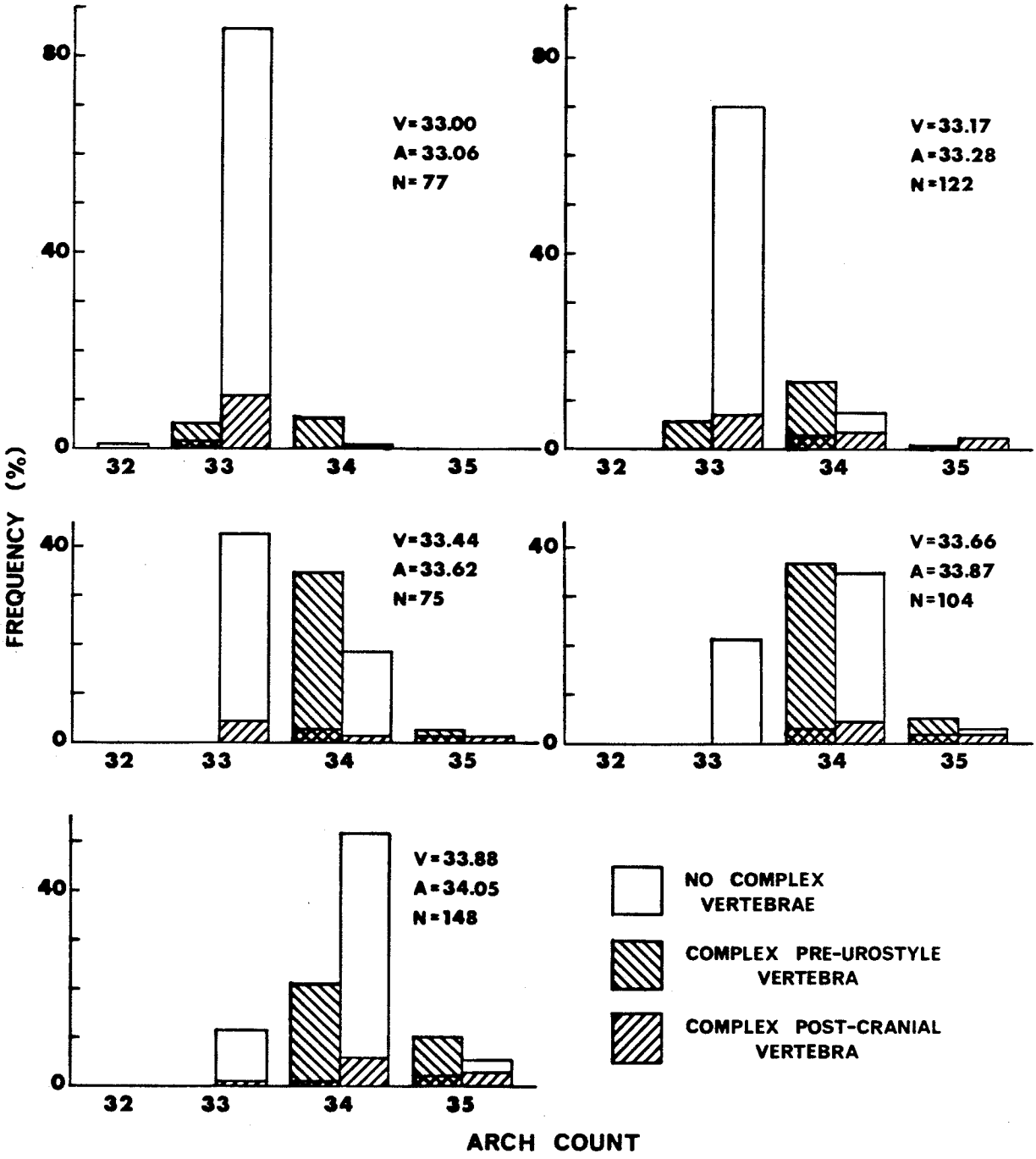
Garside (1966) argued that centra are the basic units of vertebrae and that complex vertebrae should accordingly be assigned values of "1". The basis of his argument was that the "first step in the differentiation of vertebrae is the serially discontinuous investment of the notochord by tissue of the sclerotome which forms the centra". However, Gabriel (1944) reported that in *Fundulus heteroclitus* sclerotome tissue forms a continuous perichordal sheath, only retaining segmental structure in the rudiments of the vertebral arches. According to Balinsky (1970), this is the usual course of development in vertebrates. Thus, embryological evidence argues in favor of the use of arches rather than centra as the basic indicators of vertebral segmentation.

The concentration of complex vertebrae in the pre-urostyle region has led to the suggestion that they represent secondary fusions, caused either by limited space resulting from skeletal modifications associated with the formation of the caudal fin support (Molander and Molander-Swedmark 1957) or by forces promoting the fusion of

individual elements into the urostyle (Gabriel 1944). However, localization of complex vertebrae in the pre-urostyle region would also be expected if they represent a tendency toward a fractional vertebral count. Molander and Molander-Swedmark (1957) cited the results of Barrington (1937) as proof that complex vertebrae represent the fusion of two separate elements. Barrington found that the penultimate and antepenultimate vertebral arches in *Pleuronectes platessa* and *Gadus morrhua*, respectively, are each formed from the fusion of two elements belonging to different segments. However, these vertebrae showed little or no sign of their compound origin when fully developed and would not be classified as complex vertebrae in meristic studies. The phenomenon investigated by Barrington appears more analogous to the invariable fusion of several elements to form the urostyle than to the variable formation of complex vertebrae. Thus, his results may have no bearing on the formation of the latter structures.

In *R. marmoratus*, the absence of morphological irregularity among most complex pre-urostyle vertebrae suggests that these vertebrae are fundamentally different from fusions elsewhere along the column and possibly do not represent secondary fusions of already determined segments. Thus, assigning values to complex pre-urostyle vertebrae which reflect their morphological intermediacy between one and two separate vertebrae may be appropriate. Indeed, Figure 17 shows that the frequency distributions of complex pre-urostyle vertebrae within and among samples of *R. marmoratus* are those expected if these vertebrae

Figure 17. Frequency distributions of vertebral columns with and without complex pre-urostyle and/or post-cranial vertebrae in samples with mean vertebral numbers varying between 33 and 34. V = mean vertebral count; A = mean arch count; N = sample size.


80
40
0
V=33.00
A=33.06
N=77
V=33.17
A=33.28
N=122
V=33.44
A=33.62
N=75
V=33.66
A=33.87
N=104
V=33.88
A=34.05
N=148
32
33
34
35
FREQUENCY (%)
ARCH COUNT
NO COMPLEX VERTEBRAE
COMPLEX PRE-UROSTYLE VERTEBRA
COMPLEX POST-CRANIAL VERTEBRA

represent tendencies toward intermediate counts:

1) in samples with means between 33 and 34 vertebrae, the frequency of complex pre-urostyle vertebrae is very low in vertebral columns with 33 pairs of arches and high in columns with 34 or 35 pairs of arches; and,

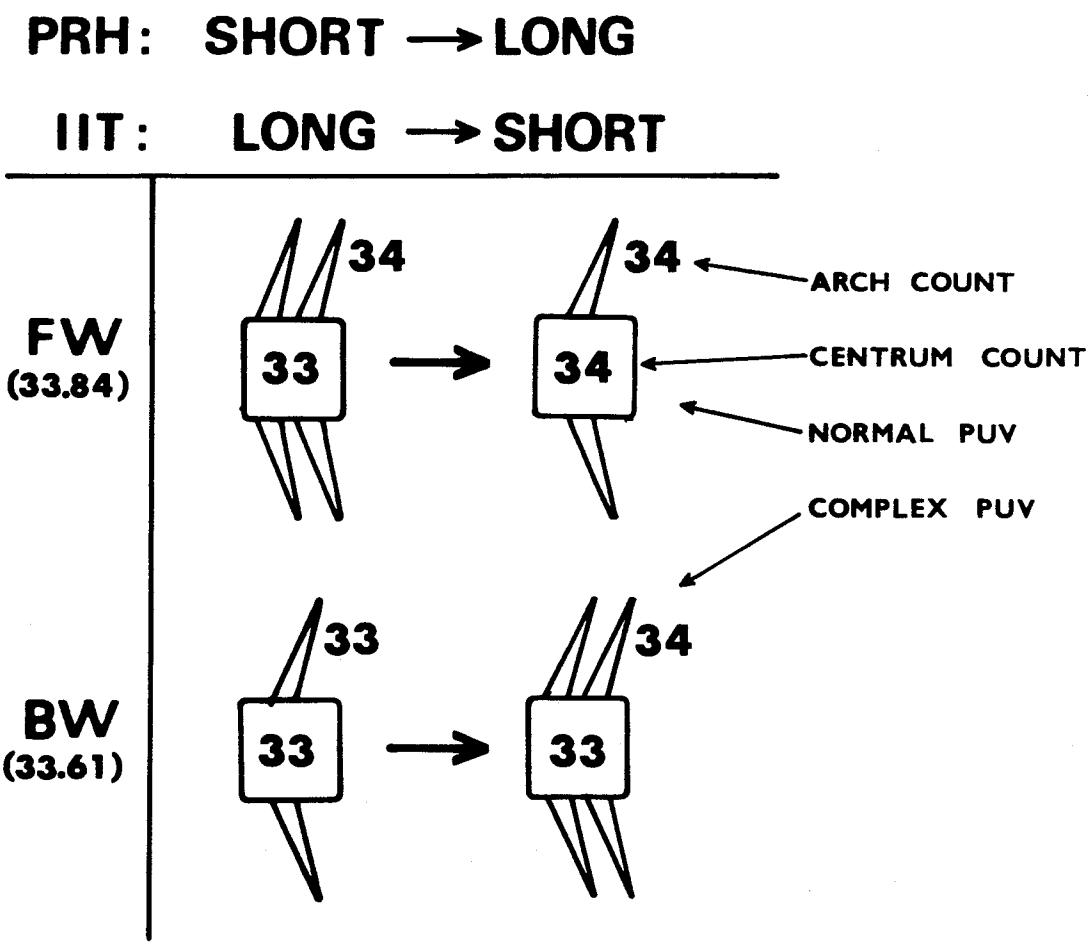
2) the incidence of complex pre-urostyle vertebrae in 34-arch columns decreases as mean vertebral number increases toward 34. A similar trend is evident in Table 11, showing Taning's (1944) samples of *Salmo trutta*.

The differences in vertebral responses to PRH and intraparental incubation time between fresh- and brackish water samples are also consistent with the suggestion that complex pre-urostyle vertebrae represent "fractional" counts. Both factors significantly influenced arch but not centrum counts in brackish water samples, and centrum but not arch counts in freshwater samples (Tables 4 and 7). Mean vertebral numbers are closer to 34 in freshwater samples than in brackish water samples (see Figure 10E). Thus, if complex pre-urostyle vertebrae reflect a tendency toward fractional counts, more variation between 34-arch columns with normal or with complex pre-urostyle vertebrae should occur in freshwater samples than in brackish water samples, and more variation between 33-arch columns and 34-arch columns with complex pre-urostyle vertebrae should occur in brackish water samples than in freshwater samples (see Figure 18).

TABLE 11. Incidence of complex pre-urostyle vertebrae in 59-arch vertebral columns in samples of trout with mean counts between 58 and 60 vertebrae (from Tåning 1944).

Mean Arch Count	N	Proportion of 59-arch columns with complex pre-urostyle vertebrae
58.2 - 58.3	49	.39
58.41 - 58.48	133	.29
58.50 - 58.59	262	.23
58.72 - 58.79	332	.15
58.82 - 58.89	435	.12
59.15 - 59.17	392	.02
59.24 - 59.27	61	.03
59.30 - 59.38	362	.01
59.49 - 59.51	98	.11
59.70	30	.03

Figure 18. Possible explanation of the different effects of parental reproductive history (PRH) and intra-parental incubation time (IIT) on arch and centrum counts in brackish (BW) and freshwater (FW) samples, assuming complex pre-urostyle vertebrae (PUV) represent fractional counts. Mean vertebral counts are shown in parentheses.


PRH: SHORT → LONG
IIT: LONG → SHORT
FW
(33.84)
34
33
34
34
ARCH COUNT
CENTRUM COUNT
NORMAL PUV
COMPLEX PUV
BW
(33.61)
33
33
34
33

In summary, assigning values of 1.0 or 2.0 to complex pre-urostyle vertebrae seems inappropriate. Patterns of variation in the frequency of these vertebrae within and among samples strongly suggest that 1.5 is a more appropriate value. In fact, the appearance of continuous morphological variation between one and two normal vertebrae at this site in the vertebral column, at least in plaice (Molander and Molander-Swedmark 1957) and *R. marmoratus,* suggests that even the assignment of values of 1.0, 1.5 or 2.0 in this region may be a discretized approximation of actual variation. However, greater knowledge of the details of vertebral development and an understanding of the mechanisms regulating somitic and vertebral segmentation are needed to decide whether the concept of fractional vertebral numbers is realistic or useful.

Complex post-cranial vertebrae are usually considered to be of minor importance (e.g. Tåning 1944, Molander and Molander-Swedmark 1957). In this study, they were considered as secondary fusions of two elements rather than tendencies toward intermediate counts, and were accordingly counted as two vertebrae, for the following reasons:

1) Any tendency toward a fractional vertebral number would probably be manifested near the caudal end of the column, not at the front where somitic differentiation occurs early in development.
2) Unlike complex pre-urostyle vertebrae, complex post-cranial

vertebrae showed no morphologically intermediate states that might suggest continuous variation between one and two vertebrae. Their neural arches and transverse processes were always completely separated. This morphology suggests secondary fusion of the centra after arches had been determined.

3) The frequency distributions of complex post-cranial vertebrae among and within samples are not those expected assuming that they represent tendencies toward intermediate counts (Figure 17): their frequencies were similar in columns with arch counts above (34) and below (33) sample means (they were, however, high in the rare 35-arch columns); no marked regular decline in their frequency occurred as the sample mean increased (except in the 35-arch columns).

While it thus seems likely that complex post-cranial vertebrae also represent secondary fusions of two vertebrae, a fundamental difference between these structures and other vertebral fusions is indicated by differences in incidence and morphology, and by the absence of an association between the former structures and stage at oviposition.

Results of meristic studies are sometimes suggested to be artifacts of counting methods. Notably, Garside (1966, 1970) suggested that the right-hand ascending limb of V-shaped vertebral responses to sustained incubation temperatures is an artifact of counting complex vertebrae as more than one vertebra. Referring to the results of

Molander and Molander-Swedmark (1957), he reported that "the increases in vertebral number at both high and low temperatures are accompanied by considerable increases in the percentage frequency (35 - 50%) of individuals with complex vertebrae" and suggested that counting these vertebrae as two vertebrae has accentuated, if not created, the V shape of the curves. Examination of their data, however, reveals that the increases in vertebral number at high temperature are in fact accompanied by a decrease of 1.3% in one case and an increase of only 7.7% in the other in the incidence of complex vertebrae. Seymour (1959) also reported V- or U-shaped vertebral curves. According to Garside (1970), complex vertebrae also occurred with much greater frequency at the extreme temperatures of incubation in the latter study. Seymour (1959) did find that "abnormal" vertebrae occurred in higher frequencies at extreme incubation temperatures but apparently did not include complex vertebrae in this category. Furthermore, Seymour (1959) apparently counted complex vertebrae as one vertebra ("In the caudal area the centrum was counted as one if separation was not complete") and still obtained U-shaped curves. In short, I find no support for Garside's suggestion that V-shaped vertebral curves are artifacts of inappropriate counting techniques.

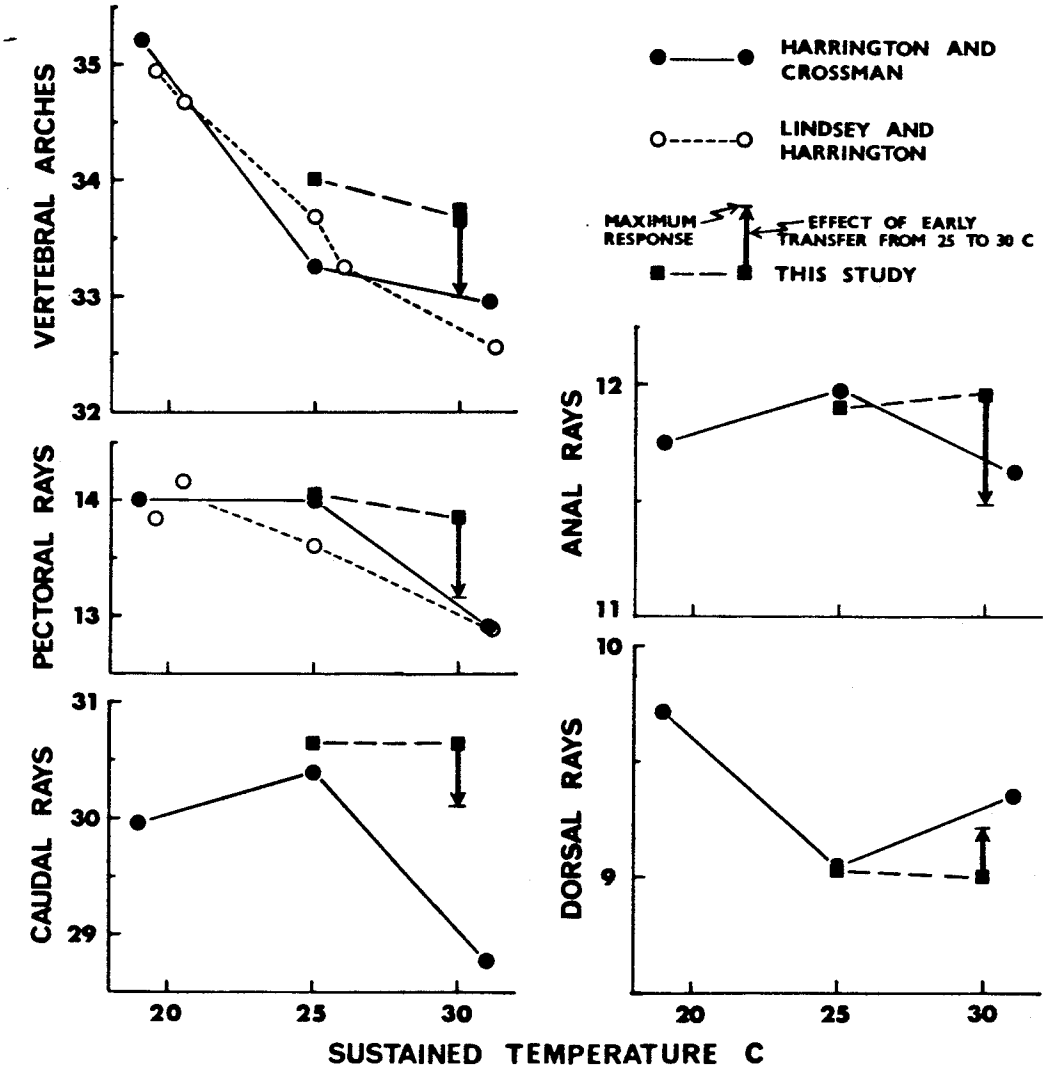
It seems likely, at least in the present study, that differences in counting methods only alter the sensitivity of analyses and cannot create spurious trends in counts. In this study, arch, centrum and "vertebral" counts always displayed similar trends.

Counting arches tended to amplify vertebral variation if means were low in the range between 33 and 34 vertebrae, while counting centra tended to amplify variation if means were high in this range. The sensitivity of "vertebral" counts to treatment effects appeared to be relatively independent of sample means, a further argument in favor of the latter counting method.

4.2 Meristic Plasticity and Genetic Uniformity in *R. marmoratus*

In Figure 19, meristic counts produced by different incubation temperatures in this study are compared with those produced in the same clone (DS) of *R. marmoratus* in the studies of Lindsey and Harrington (1972) and Harrington and Crossman (1976a). At the intermediate incubation temperature (25C) mean fin ray counts agree closely between this study and that of Harrington and Crossman, attesting to genetic uniformity within the clone. However, Lindsey and Harrington reported relatively low pectoral ray counts among fish reared at 25C. Total vertebral numbers produced by incubation at 25C differed among all three studies. The cause of these differences in vertebral and pectoral ray numbers is unknown. Vertebral differences could result from differences in counting methods, but this is unlikely since the vertebral arch counts shown in Figure 19 for the present study should be equivalent to the counts made by earlier workers. Incubation salinities, daily light durations and methylene blue concentrations were similar in the three studies. Parental reproductive histories, grandparent temperature histories,

Figure 19. Mean meristic counts produced in the DS clone of *Rivulus marmoratus* by various sustained incubation temperatures in this and earlier (Lindsey and Harrington 1972, Harrington and Crossman 1976a) studies. Arrows indicate the effect of early transfers from 25C to 30C in this study.


HARRINGTON AND CROSSMAN
LINDSEY AND HARRINGTON
MAXIMUM RESPONSE
EFFECT OF EARLY TRANSFER FROM 25 TO 30 C
THIS STUDY
VERTEBRAL ARCHES
35
34
33
32
PECTORAL RAYS
14
13
CAUDAL RAYS
31
30
29
ANAL RAYS
12
11
DORSAL RAYS
10
9
20
25
30
SUSTAINED TEMPERATURE C

light intensities, intraparental incubation times and developmental stages at transfer to freshwater probably all varied somewhat among the studies and might include the sources of the differences (although variation in most of these factors might be expected to also affect median or caudal fin ray counts). Incubation temperature was slightly more variable in the study by Lindsey and Harrington, and parental temperatures were similar but not identical in the three studies; variation in these factors might also have contributed to meristic differences among the studies.

Mean counts at high incubation temperatures differed greatly between the present and earlier studies, but these differences are attributable to extralimitary responses to temperature breaks in the latter. In the early studies, embryos were incubated initially at 25 - 26C and transferred to alternate temperatures between stages 2 and 15 (Harrington and Crossman 1976a) or mostly at stages 16 - 17 (Lindsey and Harrington 1972). This procedure seemed justified since a temperature break experiment, unfortunately deficient in early transfers, had appeared to indicate a later onset of meristic thermolability, at least for vertebrae and pectoral rays (Lindsey and Harrington 1972). However, the more extensive series of early temperature breaks in the present study demonstrates that all meristic series were thermolabile at the times of transfer to experimental incubation temperatures in the earlier studies.

As shown in Figure 19, all meristic differences between the present and earlier studies at high incubation temperature are those predicted by the extralimitary responses to early transfers from 25C to 30C observed in this study.

Lindsey and Harrington (1972) and Harrington and Crossman (1976a) reported that thermally-induced vertebral variation in *R. marmoratus* exceeded that observed in other fish species, and suggested that this uniquely high thermolability might be a consequence of homozygosity. In fact, the great vertebral variation reported in their studies is evidently due, at least in part, to overcompensation in vertebral counts of fish laid at 25 - 26C and then transferred soon to other temperatures for rearing. Thus, meristic thermolability of *R. marmoratus* may be no greater than that of gonochoristic fish species. Yet, thermally-induced vertebral differences reported in *R. marmoratus* by Harrington and Crossman (1976a) greatly exceeded those produced by the same range of temperatures in the closely related but gonochoristic species, *R. cylindraceus* (Harrington and Crossman 1976b). As in the experiments with *R. marmoratus*, embryos of *R. cylindraceus* were transferred from 25 - 26C to alternate incubation temperatures early in development (at stages 2 - 13a) and so probably also displayed extralimitary meristic counts. But, since average stages at temperature transfer may have differed between the two experiments, these results do not necessarily indicate greater vertebral plasticity in *R. marmoratus*. Also, differences in mean fin ray counts of

R. cylindraceus were similar to or greater than those of *R. marmoratus*, attesting to the absence of unusually great meristic thermolability in the latter species.

The present study corroborates the small ranges of meristic variation observed within most samples of *R. marmoratus* in earlier studies. After exclusion of fish with vertebral fusions, most meristic counts lay within one unit of the mean in all samples; in many samples, variation was confined to two counts (except in "vertebral" counts), the minimum range possible to produce non-integer means. Often when means closely approached integer values, almost no variation whatever occurred in counts (e.g. pectoral ray counts in some samples). This small within-sample variation is expected in a homozygous population, but is not entirely peculiar to such populations (for example, see some vertebral frequency distributions reported by Tåning 1944, Ali and Lindsey 1974, and Dentry 1976).

4.3 Effect of Light Intensity on Meristic Counts

Several studies have demonstrated significant influences of incubation light intensity or duration on numbers of meristic parts in fishes (McHugh 1954, Vibert 1954, Lindsey 1958, Canagaratnam 1959, Ali 1962, MacCrimmon and Kwain 1969, Kwain 1975). Generalizations about patterns of meristic response to light are not yet possible. In studies involving more than two levels of sustained intensity or duration, V- or chevron shaped or more complex patterns are commonly reported, both for vertebrae and fin rays (Vibert 1954,

Canagaratnam 1959, MacCrimmon and Kwain 1969, Kwain 1975).

The few experiments involving transfers between different light intensities or durations suggest meristic responses basically similar to those elicited by temperature breaks. Canagaratnam (1959) found that transfers between different light duration regimes produced extralimitary responses in some meristic series in sockeye salmon. Although control counts for sustained incubation in darkness are not available in the present study, the significant depressions of caudal, anal and pectoral ray counts by transfers to darkness late but not early in development indicate extralimitary responses in *R. marmoratus*. The indication that transfers to darkness at early and intermediate developmental stages might alter vertebral counts in opposite directions relative to the light control suggests extralimitary responses in vertebral counts also. Eisler (1961) reported similar meristic responses to light pulses in chinook salmon. Light pulses applied at early and intermediate developmental stages altered vertebral counts of salmon in opposite directions relative to the value produced by sustained incubation in near darkness, indicating at least one period of extralimitary response to light pulses. Although responses varied somewhat between crosses, anal ray counts of salmon appeared to be decreased by light pulses at early, intermediate and late developmental stages, suggesting responses similar to those elicited in fin ray counts of *R. marmoratus* by light breaks.

Light influences might be thermal, photochemical or photoelectric

(see Eisler 1961). The involvement of different influences at different intensities might explain the apparent complexity of meristic responses to sustained intensities and daily durations of light. However, responses to light breaks and pulses during development show important similarities (extralimitary responses) to those produced by temperature breaks and pulses, suggesting that light and temperature influences on the numbers of meristic parts might involve fundamentally similar mechanisms.

4.4. Effects of Duration of Intraparental Incubation on Meristic Parts

Two independent effects on meristic parts were associated with intraparental incubation time: an increase in the incidence of vertebral fusions associated with oviposition during a certain developmental period, and a modification of counts apparently related to differences in intra- and extraparental environments. The disruption of normal vertebral development involved in the first effect may result from a shock caused by sudden environmental changes associated with oviposition, or from mechanical shock related to extrusion through the genital pore. The first possibility seems unlikely: although vertebral abnormalities can be produced in fish by brief pulses of lethal temperatures during development (Orska 1956), they do not appear to be caused by less extreme changes in levels of temperature, light or salinity (according to the results of this study and the many other temperature break and pulse experiments cited below). On the other hand, mechanical shocks applied during

certain developmental periods appear to cause vertebral abnormalities in trout (B. Fallis, pers. comm.).

The period when vertebral fusions are most likely to be induced suggests that they are associated with vertebral chondrification rather than with primary somitic segmentation. In *R. marmoratus*, vertebral fusions were most common when oviposition occurred relatively late in the vertebral labile period, but fusions occurred at any location along the vertebral column. Similar relationships occur in the medaka (Tomita and Matsuda 1961, Ogawa 1965).

The notochord is apparently involved in the regulation of normal centrum chondrification (Hall 1977). Mechanical shocks applied to fish embryos during some periods of development produce high incidences of notochordal abnormalities (Battle 1944). This could explain the disruption of normal vertebral chondrification by mechanical shocks experienced during embryonic development.

Severe vertebral fusion often appears to be associated with a slight effect on primary metameric segmentation. In the medaka, genetically or chemically induced vertebral fusions are associated with reductions in mean numbers of anal fin rays (Tomita and Matsuda 1961, Ogawa 1965). In this study, fish with severely fused vertebral columns occasionally had unusually low vertebral arch and fin ray counts, and mean meristic counts of fish with vertebral fusions tended to be lower than those of normal fish.

The significant regressions between intraparental incubation time and some meristic counts in the brackish water series presumably

reflect effects of transfers between different intra- and extra-parental levels of one or more environmental factors. The relations between meristic counts and laying time are probably not linear, at least if mechanisms similar to those involved in temperature, light and salinity effects are operating. However, no other pattern of response is apparent in counts grouped by estimated stage at oviposition (although sample sizes were small and transfer effects apparently weak). The effective environmental factors are unknown. Levels of light intensity, salinity, oxygen and carbon dioxide all probably vary slightly between intra- and extraparental environments, and all are known to affect meristic counts in some species. Dorsal, caudal and possibly pectoral ray counts were apparently unaffected by intraparental incubation time even though they were significantly affected by experimental transfers between salinites and/or light intensities. This suggests that salinity and light intensity are not involved in the effect of intraparental incubation time.

4.5 Effect of Incubation Salinity of Meristic Counts

Experimental investigation of the influence of incubation salinity on meristic counts is sparse. Transfer from brackish to freshwater during development significantly influenced numbers of vertebrae and fin rays in *R. marmoratus*. Effects of sustained incubation salinities on some meristic counts have also been demonstrated in most other experimental studies of salinity effects (Table 12).

TABLE 12. Experimental studies of effects of incubation salinity on meristic counts.

Author	Species	Results
Heuts 1949	*Gasterosteus aculeatus* (threespine stickleback)	Depending on race and incubation temperature, anal, dorsal and pectoral ray counts increased, decreased or remained about the same with increasing salinity. More complex patterns are possible since only two salinities were used in most cases.
Hempel and Blaxter 1961	*Clupea harengus* (herring)	Relationship between myotome counts and incubation salinity was either aclivous (authors' interpretation) or chevron-shaped (my interpretation for sustained salinities).
Ali 1962	*Oryzias latipes* (medaka)	Freshwater incubation produced fewer vertebrae than incubation at a salinity of 25.9‰. Fin ray counts were not significantly affected by salinity.
Lindsey 1962	*G. aculeatus* (threespine stickleback)	Complex patterns of response. May be V-shaped for caudal rays and dorsal and anal ray basals.
Fonds *et al.* 1974	*Belone belone* (garfish)	Vertebral number tended to decrease slightly with increasing salinity between 10 and 40‰.
Fahy and O'Hara 1977	*Fundulus majalis* (a killifish)	No significant effects on vertebral count.
this study	*Rivulus marmoratus*	Transfer from brackish to freshwater during development increased meristic counts.

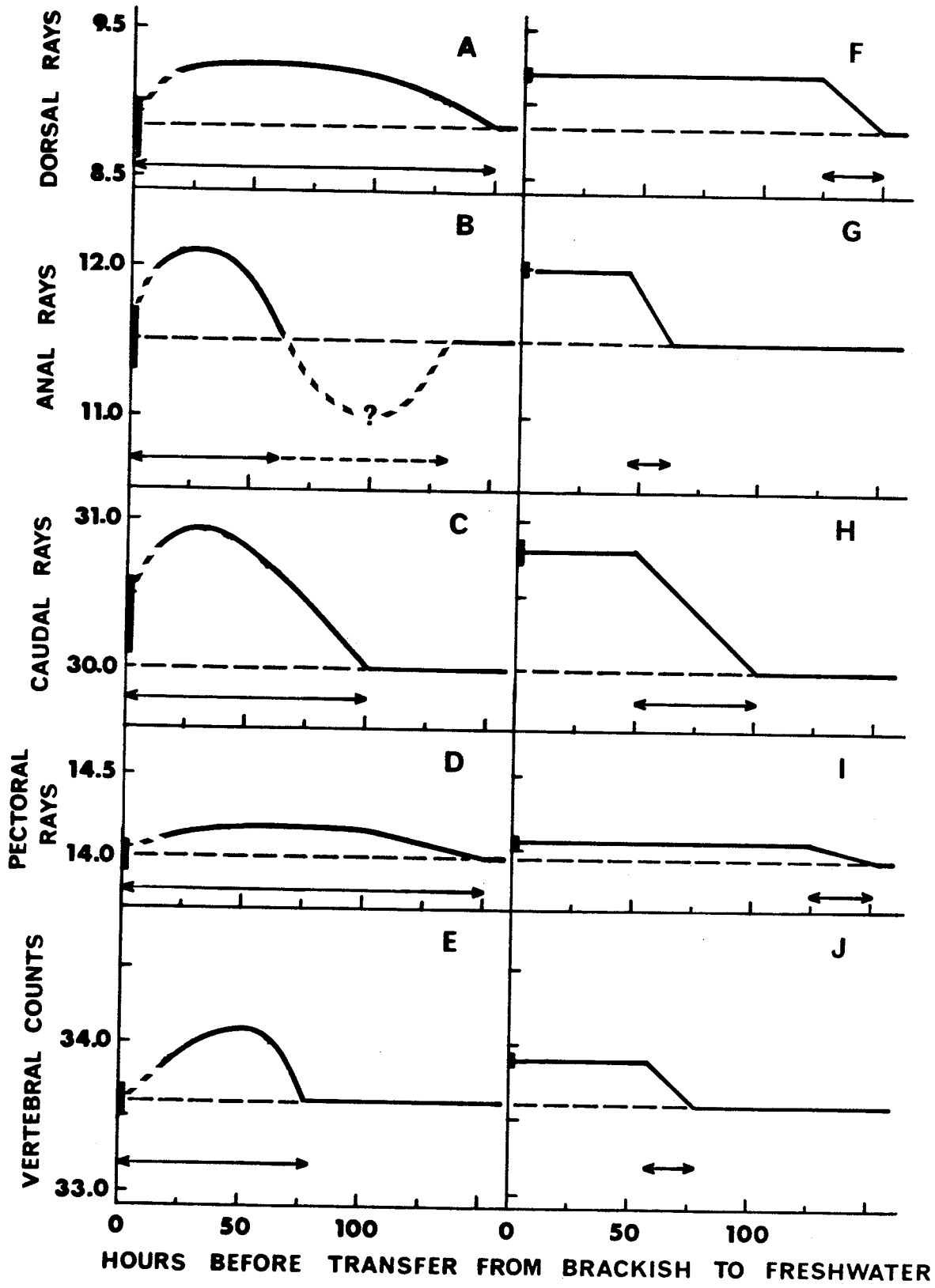
No generalizations regarding patterns of response to varying sustained incubation salinity are apparent, except that they may be complex (V- or chevron shaped).

Fahy and O'Hara (1977) found no significant differences among the mean vertebral counts of *Fundulus majalis* reared in different salinities from gastrulation to hatching. They noted that the permeability of *Fundulus* eggs decreases within the first hour after fertilization and implied that a permeability barrier formed before gastrulation may explain the absence of salinity effects in their experiment. This explanation is unlikely unless the formation of chorion permeability barriers is peculiar to *Fundulus*; significant salinity effects occurred in *R. marmoratus* embryos transferred between salinities many hours after fertilization. Since Fahy and O'Hara pooled gametes from several males and females, genetic variation may have obscured effects of incubation salinity in their experiments.

The present investigation is the only study known to me in which embryos were transferred between salinities at several developmental stages. Due to the lack of control counts for sustained incubation in freshwater, most meristic responses to "salinity breaks" in this study are consistent with two possible interpretations:

1) brief labile periods late in development, with freshwater controls above the brackish water values (as suggested by panels F-J in Figure 20); or,

Figure 20. Alternate interpretations of meristic responses to salinity breaks (see text). Heavy lines show counts produced by transfers from brackish to freshwater at indicated developmental times. Broken horizontal lines show counts produced by sustained incubation in brackish water. Vertical bars encompass probable counts which would be produced by sustained incubation in freshwater. Arrows delimit periods of meristic lability to salinity.


DORSAL RAYS
9.5
8.5
A
F
ANAL RAYS
12.0
11.0
B
?
G
CAUDAL RAYS
31.0
30.0
C
H
PECTORAL RAYS
14.5
14.0
D
I
VERTEBRAL COUNTS
34.0
33.0
E
J
0
50
100
0
50
100
HOURS BEFORE TRANSFER FROM BRACKISH TO FRESHWATER

2) prolonged periods of lability similar to those delimited by temperature breaks, and extralimitary responses to breaks during labile periods, with freshwater controls near the brackish water values (panels A-E in Figure 20).

The slight suggestion of dome-shaped responses for vertebrae and possibly pectoral rays (Figure 10) favors the second interpretation.

If the second interpretation is correct, meristic responses to salinity breaks are of the type described by the meristic response model of Lindsey and Arnason, suggesting that incubation salinity might affect meristic counts via differential effects on the rates of two processes such as growth and differentiation. Demonstrations that salinity may affect rates of embryonic development (Kinne 1964, Forrester and Alderdice 1966) indicate that this mechanism is at least possible. However, effects of salinity on developmental rate appear to be more complex than temperature effects: increasing salinity may accelerate, retard or have no effect on the rate of development (Kinne 1964, Forrester and Alderdice 1966, Fonds *et al.* 1974). This might produce meristic repsonses more complex than those elicited by incubation temperature.

4.6 Meristic responses to Temperature Transfers During Development

Meristic responses to temperature breaks and pulses during development have been studied extensively (Tåning 1944, 1952; Lindsey 1954; Orska 1956, 1962, 1963; Molander and Molander-Swedmark 1957; Hempel

and Blaxter 1961; Ali 1962; Ogawa 1971; Lindsey and Harrington 1972; Fahy 1972, 1976; Ali and Lindsey 1974; Hallam 1974). However, this wealth of experimentation has produced few generalizations and many apparent contradictions. This is particularly true for fin ray responses, which have received relatively little attention compared to vertebral responses and appear to be more sensitive to extraneous influences (Täning 1944, Ali and Lindsey 1974). Many of the apparent contradictions in reported periods and patterns of response probably stem from the long intervals between transfers and the restricted ranges of developmental stages at transfer used in many of the above studies. Only the present study and the experiments reported by Orska (1962, 1963), Ali (1962), Ali and Lindsey (1974) and Hallam (1974) include transfers over a wide range of developmental stages.

Results of the present study indicate prolonged periods of thermolability beginning at about fertilization both for vertebrae and fin rays in *R. marmoratus*. Lindsey and Harrington (1972) reported fixation times for vertebrae and pectoral rays in *R. marmoratus* similar to those indicated in this study, but provided no information regarding the onset of lability in any meristic series. Although no other investigations include temperature transfers between fertilization and late blastula stages, those that do include a wide range of transfer times suggest similar prolonged thermolabile periods for vertebrae in other fishes (medaka: Ali and Lindsey 1974; trout:

Orska 1962, Hallam 1974). However, evidence for early and extended thermolability in fin ray counts of other fishes is more scarce. Hallam (1974) did not investigate fin ray responses. Pectoral ray responses to temperature breaks reported by Ali and Lindsey (1974) are consistent with thermolability from about fertilization to shortly before hatching in medaka. But responses of other fin ray series in medaka were more erratic and appeared to suggest later onsets of thermolability (Ali 1962, Ali and Lindsey 1974). Responses in these series may however have been complicated by variation due to extraneous factors (Ali and Lindsey 1974). Orska (1963) reported that dorsal and anal ray counts in trout are labile to temperature pulses for an extended period from or before gastrulation to about the beginning of retinal pigmentation.

In contrast to the above, Molander and Molander-Swedmark (1957) and Ogawa (1971) concluded that vertebral counts of plaice and medaka, respectively, are thermolabile during brief periods relatively late in development. However, considering the complex patterns of vertebral responses to temperature breaks, these studies employed insufficient numbers and ranges of transfer times to warrant such conclusions. Tåning (1952) also suggested relatively brief and late periods of meristic lability to temperature breaks. He stated that the thermolabile periods of fin rays in trout began late in development, after the beginning of retinal pigmentation (at about 160 to 215 day-degrees (D^{o})) and apparently suggested two relatively brief

periods of vertebral sensitivity to incubation temperature:

1) a period from 40 - 53 D^{o} to 90 - 100 D^{o} (equivalent to about stages 10 to 14-17 in this study) when vertebral count was ordinarily determined (Tåning 1946, 1952); and,

2) a "supersensitive" period from about 145 to 165 D^{o} (about stages 21 - 24 to 25) when temperature transfers produced striking paradoxical reactions (Tåning 1944, 1946, 1952).

However, since Tåning (1946, 1952) did not report actual counts nor transfer times for his experiments at early developmental stages, his conclusions cannot be evaluated in the light of the complex patterns of response revealed by recent studies.

Fahy (1976) concluded that vertebral count is fixed relatively early in development (at about the equivalent of stage 19 in this study) in *Fundulus majalis*. This conclusion must be viewed with caution since some later transfers in his experiments produced an insignificant paradoxical reaction, and significant variation may have been obscured by the pooling of gametes from several parents and transfer of embryos to starting experimental temperatures late in epiboly.

The results of the present study do not support the suggestion that the "phenocritical" periods of different meristic series occur roughly in the order of their visible ontogenetic appearance (Tåning 1944, Ali and Lindsey 1974). However, they do suggest that the fixation times of different meristic series might reflect this order.

Vertebral responses to temperature breaks follow an apparently consistent pattern: breaks early in development produce overcompensation (Ali and Lindsey 1974, Hallam 1974, this study) and/or those late in development produce paradoxical reaction (Tåning 1944, Ali and Lindsey 1974, Hallam 1974). In one out of seven experiments, Hallam (1974) reported a paradoxical reaction in one lot transferred between temperatures early in development. He suggested that vertebral responses to temperature breaks may include three periods of alternating extralimitary responses. However, the lot showing the apparent paradoxical reaction was also exposed to extraneous environmental stress shortly after transfer. The occurrence of overcompensation in most lots transferred at this time in Hallam's other temperature break series, and the absence of three periods of extralimitary vertebral response in all other reported temperature break experiments, suggest that the extraneous stress may have been the cause of the apparent early paradoxical reaction reported by Hallam.

Since only this study and that of Ali and Lindsey (1974) report fin ray responses to temperature breaks over a wide range of developmental times, generalizations regarding patterns of fin ray response may be premature. The available evidence suggests that patterns vary among fins. Like vertebrae, pectoral ray counts appear to respond with early overcompensation and/or late paradoxical reaction, at least in *R. marmoratus* (Lindsey and Harrington 1972, this study) and possibly in medaka (Ali and Lindsey 1974). Caudal ray responses also resembled vertebral responses in *R. marmoratus,* but apparently

not in medaka, though responses in the latter may have been complicated by extraneous influences (Ali and Lindsey 1974). In median fins, patterns of response may be reversed compared to vertebral responses, involving an early paradoxical reaction and late overcompensation (e.g. dorsal rays in this study and probably anal rays in Lindsey 1954), or may consist of a single prolonged period of paradoxical reaction (e.g. anal rays in this study). The anal ray response reported by Ali and Lindsey (1974) in medaka apparently included three periods of alternating extralimitary responses but may have been influenced by extraneous factors. In all cases cited, the classification of the extralimitary responses of median fin rays may be questioned since counts produced by sustained incubation at either temperature were not significantly different. However, the apparent differences in response patterns between median fins and vertebrae and pectoral and caudal fins may reflect basic differences in the timing of processes underlying the responses (more specifically, of the "D" or differentiation process proposed in the meristic response model of Lindsey and Arnason: see Section 4.7 and Figure 21).

Responses to reciprocal breaks between the same two temperatures appear to be mirror images, both for vertebrae (Ali and Lindsey 1974, this study) and for fin rays (this study). However, patterns of response to breaks between different pairs of temperatures may be dissimilar in detail for a given meristic series in a given species. For example, in pectoral ray counts in *R. marmoratus,* breaks between

25C and 30C elicit striking overcompensation in lots transferred early in development but apparently little or no paradoxical reaction in lots transferred late (this study), while breaks between 26C and 20C produce marked paradoxical reaction in late-transferred lots (Lindsey and Harrington 1972). Such results are not necessarily contradictory: the meristic response model of Lindsey and Arnason adequately fits both sets of data using compatible parameter values (C.C. Lindsey, pers. comm.).

Generalizations regarding meristic responses to temperature pulses are not yet possible. Orska (1962) and Hempel and Blaxter (1961) reported patterns of response in vertebral and myotome counts, respectively, to temperature pulses which are basically dissimilar to all reported responses to temperature breaks. Such a difference is expected if meristic responses result from the actual temperature experienced by embryos rather than from "shock effects" due to temperature changes. Hallam (1974) suggested that vertebral responses to temperature breaks and pulses are mirror images and that vertebral counts respond to the return but not the initial transfers in pulse experiments. I find no support for this suggestion. For example, Tåning (1952) found that both pulses and breaks during the "supersensitive" period produced paradoxical reaction in vertebral counts.

Extralimitary responses are sometimes termed "shock effects" and are presumed to result from complex metabolic upsets associated with transfers between temperatures (e.g. Hallam 1974). This

explanation of extralimitary responses seems unlikely. Taning (1952) found that very brief temperature pulses during the "supersensitive" period had no effect on vertebral counts, suggesting that the paradoxical reaction produced by longer pulses during this period resulted from exposure to the pulse temperature rather than from changes in temperature. It is difficult to imagine metabolic upsets producing the diverse patterns of response observed in temperature break and pulse experiments. In contrast, the meristic response model of Lindsey and Arnason offers a simple explanation of these complex and diverse patterns of response, assuming that the causal factor is the actual temperature experienced by developing embryos rather than temperature changes.

4.7 Embryonic Mechanisms Underlying Meristic Variation

The embryonic mechanisms underlying meristic segmentation have been probed from two complementary points of view. Meristic studies of fishes have emphasized variation in the numbers of parts and have sought an understanding of the processes generating this variation. Embryologists working with "higher" vertebrates have been impressed by the regulative nature of meristic (i.e. somite) formation and have examined how somite spacing is controlled to produce a presumably constant number of parts. However, environmentally-induced meristic variation is not peculiar to fishes; it has also been demonstrated in all classes of higher vertebrates (amphibians: Orska and Imiolek 1962, Lindsey 1966; reptiles: Fox 1948; birds: Lindsey and Moodie 1967, Orska *et al.* 1973; mammals: Lecyk 1965). Thus, it seems possible that

fundamentally similar mechanisms may control meristic segmentation in all vertebrate classes. The morphogenetic details of embryonic development admittedly vary greatly among (and within) the vertebrate classes, particularly with respect to gastrulation. However, the fundamental mechanisms governing embryonic development appear to be similar in all vertebrates (see Oppenheimer 1947; Rudnick 1955; Wolpert 1969, 1971). Accordingly, evidence from most vertebrate classes will be marshalled in the discussion below.

In the meristic response model of Lindsey and Arnason, incubation temperature (or any other environmental factor) is proposed to differentially affect the rates of two processes: the "E" process, originally presumed to be embryo growth or elongation; and the "D" process, originally presumed to be some aspect of "differentiation". The final number of meristic parts is proportional to the level attained by the "E" process (e.g. embryo length) when the "D" process has reached "a certain threshold level" at which meristic segmentation ceases (Arnason *et al.* 1978).

The meristic response model provided adequate fits to all meristic responses to temperature breaks observed in the present study. This suggests that the same basic mechanisms, perhaps modification of the rates of some aspects of embryo growth and differentiation, are involved in the influence of incubation temperature on both vertebral and fin ray counts. Responses to light and salinity breaks in this study suggested that similar mechanisms might also be involved in the effects of these environmental factors on meristic counts.

Fundamental to the original interpretation of the meristic response model is the assumption that the number of meristic parts is dependent upon the length of tissue to be segmented (that is, the length of somitic mesoderm or the width of fin buds). Some evidence suggests however that somite segmentation is at least partly size regulative. *Xenopus* larvae surgically reduced to little over two-thirds control length by the removal of animal-vegetal sectors at the blastula stage usually have somite numbers similar to those of sibling controls of the same developmental stages (Cooke 1975). Cooke (1977) reported, without details, that there are strong indications of similar regulation in bird embryos. Mutant mouse embryos one-half normal size have the same number of somites as normal embryos, at least until the tail bud stage (Flint *et al.* 1978). The regulative ability of fish embryos with regard to somite formation has not been tested. The apparent absence of a causal connection between vertebral count and teleost egg size (Lindsey and Ali 1971; C.C. Lindsey and G.B. Ayles, unpublished data) suggests some regulation to initial embryo size. Tung and Tung (1944) raised *Carassius* embryos from egg fragments, isolated blastomeres and fused eggs. These embryos were one-half to double the usual size but were otherwise normal. Tung and Tung reported no somite counts but their Figure 16, even if only approximately accurate, suggests that somite number was roughly constant over a four-fold increase in embryo size and that therefore some regulation to embryo size had occurred.

The present interpretation of the meristic response model might not be inconsistent with the apparent lack of correlation between vertebral or somite count and initial embryo size provided growth is interpreted in terms of cell numbers rather than tissue length. Embryos of different initial sizes might contain similar numbers of cells of different sizes, thus producing similar meristic counts if spacing is measured out in numbers of cells, not length of tissue.

This interpretation of the model may be reconciled with other evidence for size regulation in somite formation by supposing that the latter is controlled in two fundamentally different ways during successive periods of embryogenesis. In general, embryonic development appears to be initially regulative but finally determinate in vertebrates. For example, at the 2- and 4-celled stages, *Fundulus* embryos may regulate 50% loss (i.e. the removal of 1 or 2 blastomeres) completely, but these embryos lose most regulative ability by gastrulation (Nicholas and Oppenheimer 1942). A similar situation appears to exist in the control of somite segmentation. Although somitogenesis is apparently regulative after the removal of cells early in development, somite formation in the tail bud appears to be non-regulative. In *Xenopus*, tail bud tip removals produce truncated axes with reduced numbers of somites identical in length and shape to those of the same numerical position in unoperated siblings (Cooke 1975);

the more tissue that is removed at a given age (or the earlier it is removed) the shorter the axis and the less complete the somite complement (Cooke 1975, 1977). The observation that myotome counts per embryonic axis are inversely correlated to degree of twinning in trout (Garside and Fry 1959) suggests a similarly non-regulative system late in development in fishes.

Thus, the following system of control of somite segmentation may be suggested. The "wavelength" of initial somite (vertebral) segmentation (i.e. the number of cells forming or destined to form a somite) may be adjusted to compensate for changes in embryo size early in development, but is fixed by about the time of gastrulation. By this time, cells are allocated, in proportion to the total number of pre-somitic cells, to one of the anterior somites or to a reservoir of yet undetermined cells destined to form the tail bud and posterior somites. The number of somites determined at this time need not be great. For example, in *Xenopus*, cells destined to form the first 6 or 8 somites alone occupy most of the dorsal mesodermal mantle at the close of gastrulation, while the remaining 30 or more somites are produced from the reservoir of undifferentiated tissue incorporated into the tail bud (Cooke 1977). This initial size regulation could be produced by a system involving "positional information", whereby a cell's position within the system with respect to the two ends is uniquely specified and this information is used to determine its differentiation (for possible mechanisms see Wolpert 1969, 1971). The possible nature of such a system is unknown, but the gradients in cell behaviour

within the teleost blastoderm during epiboly (Ballard 1973a, b) suggest the existence of some such system.

The "wavelength" of segmentation in the presumptive tail bud mesoderm may or may not be regulated to changes in embryo size before gastrulation. Such regulation is probably not necessary to explain the results reported by Cooke (1975) or Flint *et al.* (1978). At any rate, assuming a fixed wavelength of segmentation after gastrulation, the final number of somites would then depend upon growth in the presumptive tail bud mesoderm before the end of segmentation, as postulated by the meristic response model.

The suggestion that similar mechanisms operate in the environmental modification both of vertebral and fin ray counts seems highly plausible if most variation in vertebral count results from influences on the development of the tail bud. Patterns of growth and differentiation are basically similar in vertebrate tail and limb or fin buds. As in the tail bud, skeletal development in limb buds appears to be non-regulative with respect to tissue addition or removal, along both disto-proximal and antero-posterior axes (Summerbell *et al.* 1973, Hampé 1958).

A pattern of somite determination which is initially but not finally regulative might be expected to produce greater environmentally-induced variation in caudal vertebral counts than in abdominal counts. This is in fact observed in many studies (.e.g this study, Ali and Lindsey 1974) but is not invariably true (e.g. Lindsey 1962). However, variation in abdominal vertebral counts could be due to variation in the number of posterior abdominal vertebrae (which are possibly undetermined at the time of final size regulation) or,

more probably, to shifts in the position of the division between the abdominal and caudal regions of the vertebral column.

The mechanisms of environmental modification of meristic counts suggested above require mechanisms of segmentation with the following properties:

1) The mechanisms controlling segmentation must be initially regulative but finally non-regulative, at least for somites. (The initial regulative phase might not occur in the determination of fin ray counts.)

2) The "wavelength" of segmentation should be insensitive to environmental conditions such as temperature (although environmentally-induced variation in the wavelength of segmentation might conceivably be absorbed in the scaling parameter of the "E" curve in the meristic response model (Arnason *et al.* 1978)).

Two basic types of mechanisms have been proposed for meristic segmentation. The earliest approach postulates "prepatterns" involving serially repeated concentration peaks of some "morphogenetic" substance (Turing 1952, Wilby and Ede 1975, Flint *et al.* 1978). The second approach involves a positional information gradient and systems of entrained intracellular oscillators (Goodwin and Cohen 1969, Cooke and Zeeman 1976). The second approach seems more compatible with the mechanisms proposed above for environmental effects on meristic counts. Unlike the prepattern models, positional

information models are readily adapted to a system of somitogenesis which is initally regulative and then non-regulative. In the prepattern models, the wavelength of segmentation depends upon rates of synthesis and destruction of the morphogenetic substance and thus is sensitive to environmental conditions. In contrast, the segmentation wavelength in the model proposed by Goodwin and Cohen (1969) is unaffected by environmental changes which slow down or accelerate cellular activity. Meristic spacing might also be independent of environmental conditions in Cooke and Zeeman's model, but this is unlikely unless the oscillator proposed in this model is also involved in measuring out rates of intracellular development. The positional information models also seem more consistent with the effects of surgical manipulation of chick and amphibian embryos (Deuchar and Burgess 1967; Menkes and Sandor 1977; Packard 1978) and with the effects of heat shocks on *Xenopus* embryos (Elsdale *et al*. 1976; Cooke 1978).

4.8 Possible Prefertilization Effects on Meristic Counts

Prefertilization effects on embryonic development are strongly indicated by the influences of parental temperature and reproductive histories and possibly grandparent temperature history on meristic counts in this study. Examples of each of these types of prefertilization influence on offspring characteristics are known for

other organisms. Parental temperature apparently affects some offspring meristic counts in the zebrafish *Brachydanio rerio* (Dentry 1976, Dentry and Lindsey 1978), early thermal tolerance of embryos in the silverside *Menidia audens* (Hubbs and Bryan 1974), and the rate of development to hatching in *Drosophila* (Edney 1969). Parental age influences the survival of progeny from eyed eggs to the time of yolk sac absorption in some salmonids (Ayles and Berst 1973) and a wide variety of offspring characteristics in *Drosophila* and other invertebrates (Lints and Hoste 1976). An effect of grandparent temperature is not known in any other organism, but parental age effects may accumulate over several generations in *Drosophila* (Lints and Hoste 1976) and grandparent age at the time of parental birth affects the production of winged offspring in aphids (MacKay and Wellington 1977). Other "prefertilization" effects reported in fish include:

1) a nonheritable effect of parental diet on the incidence of vertebral deformities among progeny in the medaka *Oryzias latipes* (Tomita and Matsuda 1961),
2) an increase in notochordal abnormalities among *Gasterosteus aculeatus* produced from eggs subjected to mechanical shock before fertilization (Battle 1944),
3) an increase in vertebral counts and irregularities among *Salmo trutta* produced from eggs fertilized some hours after maternal death (Tåning 1944), and
4) an effect of parental exposure to X-irradiation about 3 months prior to spawning on survival, incidence of deformation and

growth rates of offspring in *Salmo gairdneri* (Foster *et al.* 1949).

In the studies by Hubbs and Bryan (1974) and Dentry (1976), the effects attributed to parental temperature history were observed in experiments involving transfers of embryos between temperatures early in development. On the basis of an experiment involving double temperature transfers, Hubbs and Bryan concluded that a spurious effect due to temperature shock was unlikely in their study. In Dentry's study, the influence of parental temperature on vertebral number was usually in the direction predicted for overcompensation due to transfer from parental to incubation temperatures during development. However, an appreciable effect due to temperature transfer is extremely unlikely in Dentry's study due to the very early stage (the one-cell stage) at transfer in his experiments. The parental temperature effects reported in this study and by Edney (1969) cannot be attributed to temperature transfers during development since they were observed in experiments involving no such transfers.

Because all embryos are retained within parents at least briefly in *R. marmoratus,* two possible sources exist for the prefertilization effects observed in this species. The effects might result from differences in the eggs and/or sperm of parents with different temperature or reproductive histories, or from an influence of the intraparental environment experienced during early development. The latter source is not possible in the examples cited above in other species, and is also unlikely for the prefertilization effects observed in *R. marmoratus*.

There is no indication that the extent of any prefertilization influence in *R. marmoratus* varies with duration of intraparental incubation (although sample sizes were usually insufficient to directly test this possibility; however, see Appendix E). If effects of parental temperature or reproductive history result from environmental differences within the oviducts of warm vs. cold acclimated or young vs. old parents, the level of the hypothetical environmental factor involved must also vary between the extra-parental environment and at least one (and probably both) of the two supposed types of intraparental environments. Thus, effects comparable in magnitude to the prefertilization effects should attend laying at some times during meristic labile periods (if the hypothetical environmental factor influences meristic counts in the same manner as all other environmental factors studied appear to). Sample sizes were insufficient to examine this question among offspring with parents at 30C. However, among offspring of parents at 25C, effects associated with stage at laying were usually slight compared to possible prefertilization effects. Finally, the meristic series affected strongly by parental temperature or reproductive history and by intraparental incubation time do not completely correspond: for example, anal ray counts were influenced by stage at laying but not by parental temperature, while pectoral ray counts were greatly affected by parental temperature but not by stage at laying. These results argue against the likelihood that the "prefertilization" effects observed in *R. marmoratus* are due to differences in intra-

parental environments.

Thus, although conclusive proof awaits counts from fish produced from eggs fertilized artificially, the "prefertilization" effects on meristic counts in *R. marmoratus* are most likely due to differences between the eggs and/or sperm of parents of different temperature or reproductive histories. Such effects are not surprising. In a wide variety of organisms, development from fertilization to the high blastula appears to be controlled by mRNA synthesized and, in at least one case, translated during oogenesis (Davidson 1968, Brothers 1976). The teleosts are apparently no exception to this rule (Wilde and Crawford 1966; Kafiani 1970). It seems reasonable that parental reproductive condition or environmental conditions experienced during oogenesis could influence the synthesis and storage or translation of this mRNA in the oocyte. Similarly, the synthesis and storage or translation of mRNA during oogenesis could, in turn, be influenced by a chain of events affected at some point by mRNA synthesized in the egg from which an individual was produced, resulting in effects of grandparent age or temperature history. (In *Drosophila,* an individual's fecundity is influenced by maternal age, presumably via processes affected by mRNA synthesized in the egg from which it was produced (Lints and Hoste 1976)).

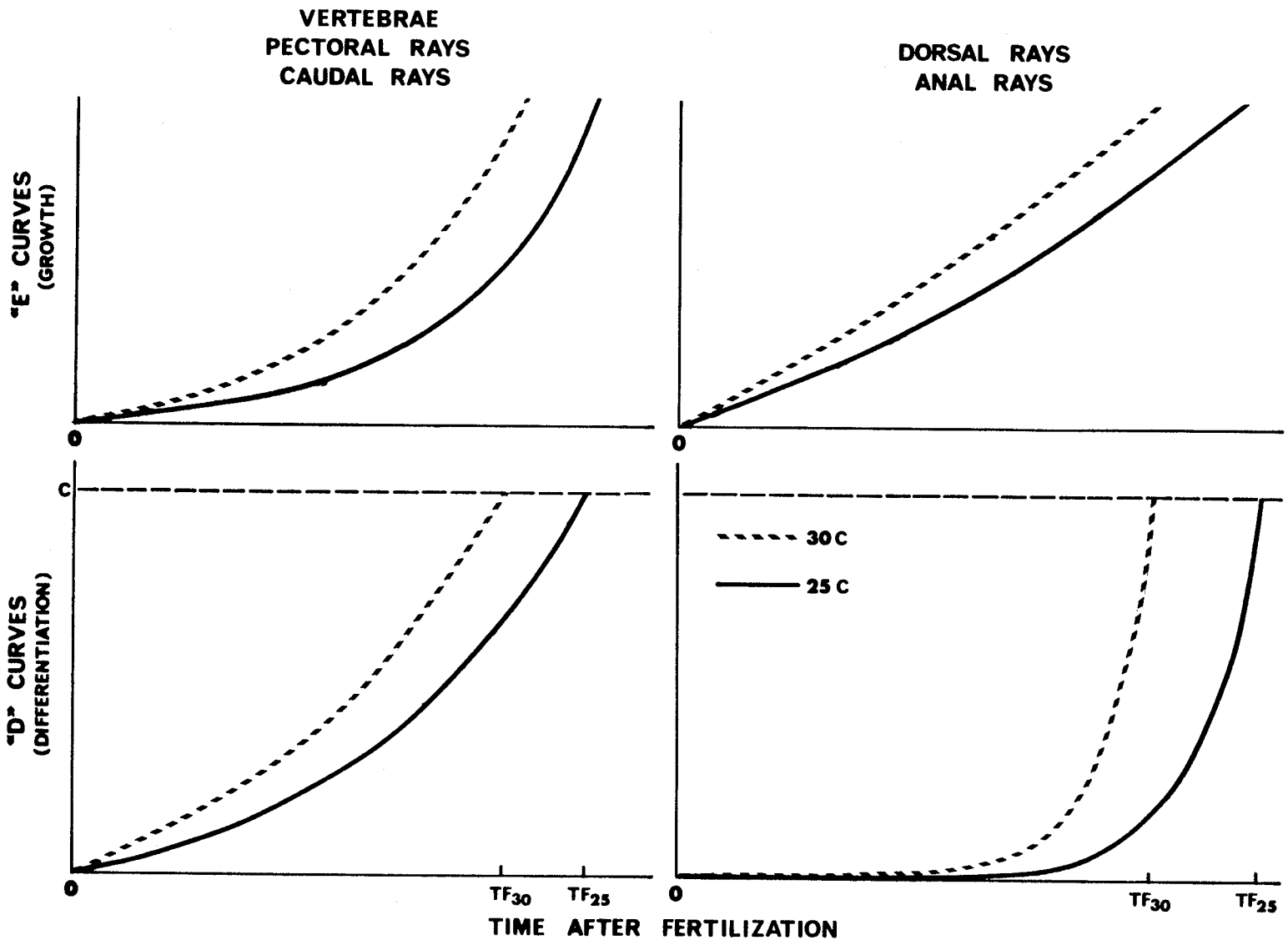
Meristic responses to parental temperature, as to incubation temperature, appear to vary among species and possibly among genotypes within a species. Those meristic series affected by parental temperature, and the direction of their responses, varied between *R. marmoratus* and the zebrafish and, in some series, between zebrafish

genotypes (Dentry 1976). Also, the effect of a new parental temperature was apparent in offspring laid sooner after parental temperature transfer in zebrafish than in *R. marmoratus,* possibly reflecting different relations between the times of fertilization and the prior synthesis or translation of certain mRNA during oogenesis between the two species.

Variation in the shapes of the "D" curves predicted for meristic series in *R. marmoratus* (Figure 21) suggest a possible mechanism for the modification of meristic counts by parental temperature. Dorsal and anal ray counts are not significantly influenced by parental temperature in *R. marmoratus,* and both have "D" curves with rates near zero until late in development. This may be coincidental, or might indicate an effect of parental temperature on the rate of this "differentiation" process early during development. An acceleration of this process (in proportion to its rate during early development) due to high parental temperature would decrease final vertebral and caudal and pectoral ray counts but have little effect on numbers of anal and dorsal rays. An influence on this process only early in development is consistent with a mechanism involving substances contained in the oocyte cytoplasm before fertilization.

Alternatively, parental temperature may affect rates of embryo growth or the initial setting of the wavelength of segmentation in certain meristic series only. The actual mechanism involved might be

Figure 21. Types of "D" and "E" curves predicted for meristic series in *Rivulus marmoratus* by the meristic response model of Lindsey and Arnason (C.C. Lindsey, pers. comm.). C = critical level of differentiation at which meristic segmentation ceases; TF_x = time to meristic fixation at χC (time scale varies according to meristic series).


VERTEBRAE
PECTORAL RAYS
CAUDAL RAYS
DORSAL RAYS
ANAL RAYS
"E" CURVES
(GROWTH)
0
0
C
"D" CURVES
(DIFFERENTIATION)
30 C
25 C
0
0
TF_{30}
TF_{25}
TF_{30}
TF_{25}
TIME AFTER FERTILIZATION

ascertained by measurements of developmental rates and cell counts per somite among embryos with varying parental temperature history.

Unlike parental temperature, parental reproductive history strongly influences dorsal and anal ray counts, suggesting the involvement of different mechanisms. Effects of parental age on survival of progeny in fish are sometimes attributed to differences in egg size or lipid quality (Nikolskii 1969, Ayles and Berst 1972). However, it is not likely that influences of parental reproductive history on meristic counts are related to egg size, since the latter does not seem to be causally connected to meristic counts. Changes in segmentation wavelength or rates or embryo growth due to qualitative changes in the oocyte cytoplasm seem to be more likely causes.

More study is required before an influence of grandparent temperature history on meristic counts may be confidently claimed. Vertebral differences apparently related to grandparent temperature in this study might actually have resulted from a concave relationship between vertebral number and parental reproductive history. Data are insufficient to determine the exact forms of relationships between meristic counts and parental reproductive history, but in *Drosophila* and other invertebrates quantitative traits often do vary in a nonlinear (sometimes convex, concave or sinusoidal) way in relation to parent age (Lints and Hoste 1976). The question of whether the development of an individual is influenced by the environment experienced by its grandparents is of great theoretical interest and warrants further study.

Effects of parental environments and reproductive condition or age on characters of offspring have important implications regarding mechanisms controlling embryonic development. These effects may also be important in the racial analyses of fish populations and the interpretation of experiments investigating phenotypic meristic variation. In fish, as in invertebrates, nonmeristic characteristics of offspring, such as early survival, growth rates and fecundity, may also be influenced by parental age or environments. This possibility may be of considerable ecological and evolutionary consequence and is of great import to fish culture and fisheries management.

5. SUMMARY

1. Adults from one clone of the self-fertilizing cyprinodont fish *Rivulus marmoratus* were held at 25C or 30C, and their offspring were either incubated at the parental holding temperature until after hatching or transferred to the alternate temperature after various periods of development. Ofspring of parents held in brackish water at 25C were incubated extraparentally in fresh- or brackish water, in darkness or with 14 h of artificial illumination daily.

2. Post-cranial and pre-urostyle vertebrae with double neural and/or haemal arches were classified as "complex" vertebrae. The morphology of complex pre-urostyle vertebrae and their frequency distributions within and among samples suggested that they reflected tendencies toward fractional vertebral counts and should be assigned values of "1.5". Complex post-cranial vertebrae appeared to be secondary fusions of two elements and were counted as two vertebrae.

3. Other vertebrae with two or more neural and/or haemal arches were classified as vertebral "fusions". Vertebral "fusions" were most common among fish laid at about stages 18-21 and were considered to be developmental abnormalities. Fish with vertebral fusions were exluded from analyses since they had significantly lower mean counts and higher variances than did those without fusions for most meristic series.

4. Anal ray counts tended to increase and vertebral and possibly pectoral ray counts to decrease slightly with increasing duration of intraparental incubation.

5. All meristic counts were increased by transfers from brackish to freshwater between 10 and 50 to 110 h of development at 25C (depending upon the meristic series). Due to the lack of control counts for sustained incubation in freshwater, the meristic responses to "salinity breaks" in this study are consistent with two possible interpretations: (i) brief labile periods late in development, with freshwater controls above the brackish water values; or (ii) prolonged periods of lability and extralimitary responses to temperature breaks during labile periods, with freshwater controls near the brackish water values.

6. Transfers from light to darkness before 37 h of development at 25C significantly decreased mean vertebral counts; those after 47-72 h of development significantly decreased mean pectoral, anal and caudal ray counts. Patterns of response to light breaks indicated extralimitary responses in pectoral, anal and caudal ray counts and possibly in vertebral counts.

7. Parental reproductive history was defined as the time between the collection of an egg and the first egg collection from its parent. Offspring of parents which had just begun to lay eggs had significantly fewer meristic parts (except caudal rays) than did those of "experienced" parents. In most series, numbers of parts appeared to peak or plateau among fish with parental reproductive histories of 100 days or less.

8. Fish with grandparents held at 30C tended to have more vertebrae than those with grandparents held at 25C. However, most vertebral differences attributed to an influence of grandparent temperature might have resulted from confounding with parental reproductive history if the

relation between vertebral number and the latter is concave, rather than linear or asymptotic.

9. Fish conceived soon after parental transfer from 25C to 30C had significantly more vertebrae and pectoral and caudal rays than did those conceived long after parental transfer. No significant differences in numbers of anal or dorsal rays occurred between fish conceived soon and long after parental transfer to 30C.

10. Vertebral and pectoral and caudal ray responses to temperature breaks resembled expected patterns only if offspring of parents held at 30C were assumed to have fewer parts than those of parents at 25C. These results indicate that the effect of parental temperature history noted above is due to the actual temperature experienced by parents, rather than to a shock effect related to parental temperature transfer. Responses to temperature breaks were consistent with a lack of effect of parental temperature on numbers of anal and dorsal rays.

11. Meristic counts were thermolabile from about fertilization to at least 70-80 h (vertebrae, caudal rays) or 144-192 h (pectoral, dorsal and anal rays) thereafter at 25C. All appeared to be fixed before hatching. Patterns of response to reciprocal temperature break experiments were roughly mirror images (assuming a parental temperature effect on numbers of vertebral and pectoral and caudal rays). Early temperature breaks produced an overcompensation in vertebral and pectoral and caudal ray counts. Temperature breaks applied at most times during the thermolabile period produced a paradoxical reaction in anal ray counts. Temperature breaks produced a paradoxical reaction in dorsal ray counts if applied early,

or an overcompensation if applied late.

12. The uniquely high vertebral thermolability reported in *R. marmoratus* by previous workers is evidently due, at least in part, to an overcompensation in vertebral counts of fish transferred from the parental holding temperature to other temperatures for rearing in the earlier studies.

13. The meristic response model of Lindsey and Arnason provided adequate fits to all meristic responses to temperature breaks observed in the present study. This suggests that incubation temperature may influence both vertebral and fin ray counts via differential effects on the rates of two processes, possibly embryo growth and "differentiation". The responses to light and salinity breaks observed in this study suggest that similar mechanisms might also be involved in the effects of these environmental factors on meristic counts.

14. The meristic response model of Lindsey and Arnason may be reconciled with the lack of correlation between vertebral or somite counts and initial embryo size in fishes and with evidence for early size regulation in somite formation, at least in higher vertebrates, by interpreting growth in terms of cell numbers rather than tissue length and by assuming mechanisms of somite segmentation that are initially regulative but finally determinate.

15. The incongruities between the meristic series strongly affected by parental temperature or reproductive history and those affected by intraparental incubation time suggest that the former influences result from differences in the eggs and/or sperm of parents with different

temperature or reproductive histories rather than from an influence of the intraparental environment experienced after fertilization.

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APPENDIX A. Meristic counts of fish grouped by duration of intraparental incubation.

1. Total vertebral numbers in the 25P25I-FW series.

Duration of Intraparental Incubation		Centra													Arches									Vertebral Counts														
Nom. stage	Mean time (h)	14	24	26	27	28	29	30	31	32	33	34	35	Mean	30	31	32	33	34	35	36	37	Mean	30.5	31	31.5	32	32.5	33	33.5	34	34.5	35	35.5	36	36.5	Mean
i) including fish with vertebral fusions:																																						
4-6	4.9								1	1	5	9		33.38				2	15				33.88						1	5	10							33.78
8-9	8.7										2	4		33.67					6				34.00							2	4							33.83
10-11	12.0										3	1		33.25				1	3				33.75						1		3							33.75
12	16.4					1					5	6		33.08			1	1	8	2			33.92				1		1	2	6	2						33.75
13a-b	22.6									1	3	9		33.62				3	11				33.79						3		10							33.77
13c-14	27.2					1	1		1	1	11	8	1	32.92				4	14	3	2	1	34.25						4	5	9	2	1	1	1	1		34.06
15	33.6						1				1	2	1	33.00			1	1	2			1	34.00				1		1		2							34.00
16	36.8										4	7	2	33.85				2	6	5			34.23						2	1	5	2	3					34.12
17	39.0							1			3		1	32.80				1	2	2			34.20						1	2		1	1					33.90
18	46.2	1	1	1	1	1	5		1	2	3	7	1	30.46	1	2	1	3	13	4			33.54	1	1		1	1	2	4	9	2	2					33.52
19-20	56.6		1			1	1		1	2	2	4	1	31.77				1	3	6	3		33.85			1		3		2	4	1	2					33.58
21	61.4					1		2	3	5	1	8		32.30					7	11	2		33.75					2	5	2	9	1	1					33.62
22	67.1							1	1	1	8	3	1	32.93			1	4	6	4			33.87				1		4	3	3	2	2					33.70
23-26	96.0						1	1	1	3	8	8	1	32.91				4	16	2	1		34.00					1	3	7	9	2		1				33.76
27-29	146.7							1	1		4	1		32.43				2	5				33.71						2	2	3							33.57
ii) excluding fish with vertebral fusions:																																						
4-6	5.0										4	9		33.69				1	12				33.92						1	3	9							33.81
8-9	8.7										2	4		33.67					6				34.00							2	4							33.83
10-11	12.0										3	1		33.25				1	3				33.75						1		3							33.75
12	16.4										5	6		33.55				1	8	2			34.09						1	2	6	2						33.91
13a-b	22.7										3	9		33.75				2	10				33.83						2		10							33.83
13c-14	27.5										9	7		33.44				2	12	2			34.00						2	5	7	2						33.78
15	33.6										1	2		33.67				1	2				33.67						1		2							33.67
16	36.8										4	7	2	33.85				2	6	5			34.23						2	1	5	2	3					34.12
17	38.4										3		1	33.50					2	2			34.50							2		1	1					34.12
18	45.9									1	3	7	1	33.67				1	9	2			34.08						1	2	7	1	1					33.96
19-20	56.3									1	1	4	1	33.71					6	1			34.14							2	4		1					34.00
21	61.0									2	1	8		33.55				2	8	1			33.91					1	1	1	7	1						33.77
22	66.8										6	3	1	33.50				4	5	1			33.70						4	2	3		1					33.60
23-26	94.4									2	8	8	1	33.42				3	14	1	1		34.00					1	2	6	8	1		1				33.76
27-29	148.7										4	1		33.20				2	3				33.60						2	1	2							33.50

APPENDIX A (Continued...)

2. Total vertebral numbers in the 25P25I-BW series.

Duration of Intraparental Incubation		Centra														Arches							Vertebral Counts									
Nom. stage	Mean time (h)	12	24	25	26	27	28	29	30	31	32	33	34	35	Mean	30	32	33	34	35	36	Mean	29.5	31.5	32.5	33	33.5	34	34.5	35	36	Mean
i) including fish with vertebral fusions:																																
2-9	8.5											4	3		33.43			1	6			33.86				1	3	3				33.64
10	10.6							1				5	3		32.89			4	4	1		33.67			1	3	2	2		1		33.50
11	11.7											4			33.00				4			34.00					3	1				33.62
12a	15.0								1			5	2		32.86			1	7			33.88				1	5	2				33.56
12b	19.7											3	2		33.40			2	3			33.60				2	1	2				33.50
13a-c	22.6									1		5	2	1	33.22				7	1	1	34.33					4	3		1	1	34.11
13d-14	28.8											9	8		33.47			3	13	1		33.88				3	6	7		1		33.71
15	33.4											8	8	2	33.67			3	12	3		34.00				3	5	7		3		33.86
16-17	40.0		1									3	3		32.14	1			5	1		33.57	1				3	2	1			33.21
18	46.1				1			1			2	9	3		32.38		1	5	8	2		33.69		1	1	4	3	5	1	1		33.50
19-20a	53.5	1		1		1	1				3	2	3		29.67	1		3	5	3		33.67	1		2	1	2	3	1	2		33.42
20b	57.3										2	2	2		33.00			1	4	1		34.00				1	3	1	1			33.67
21	61.4						1				2	3			31.83			2	4			33.67				2	2	2				33.50
22a	66.4									2	1	3	2		32.62			3	5			33.62			1	2	3	2				33.38
22b	70.0								1						30.00			1				33.00			1							32.50
25-26	93.4											2			33.00			2				33.00				2						33.00
ii) excluding fish with vertebral fusions:																																
2-9	8.5											4	3		33.43			1	6			33.86				1	3	3				33.64
10	10.6											5	3		33.38			3	4	1		33.75				3	2	2		1		33.62
11	11.7											4			33.00				4			34.00					3	1				33.62
12a	15.1											5	2		33.29			1	6			33.86				1	4	2				33.57
12b	19.7											3	2		33.40			2	3			33.60				2	1	2				33.50
13a-c	22.6											5	2		33.29				7			34.00					4	3				33.71
13d-14	28.8											9	8		33.47			3	13	1		33.88				3	6	7	1			33.71
15	33.4											8	7	2	33.65			3	12	2		33.94				3	5	7	2			33.79
16-17	39.8											3	3		33.50				5	1		34.17					3	2	1			33.83
18	46.3										1	8	3		33.17			5	6	1		33.67			1	4	3	3	1			33.46
19-20a	53.5										1	2	2		33.20			2	3			33.60			1	1	1	2				33.40
20b	57.4											2	2		33.50				3	1		34.25					2	1	1			33.88
21	63.0											2			33.00			2				33.00				2						33.00
22a	66.0											3	2		33.40			2	3			33.60				2	1	2				33.50
25-26	93.4											2			33.00			2				33.00				2						33.00

APPENDIX A (Continued)

3. Fin ray counts in the 25P25I-FW series.

Duration of Intraparental Incubation		Right Pectoral				Left Pectoral				Dorsal							Anal						Caudal						
Nom. stage	Mean time (h)*	13	14	15	Mean	13	14	15	Mean	5	6	7	8	9	10	Mean	8	10	11	12	13	Mean	28	29	30	31	32	33	Mean
i) including fish with vertebral fusions:																													
4-6	4.9		17		14.00		17		14.00				2	10	4	9.12			4	11		11.73			8	7	2		30.65
8-9	8.7		6		14.00		6		14.00					5		9.00			1	3		11.75			4		1		30.40
10-11	12.0		4		14.00		3	1	14.25				1	2	1	9.00			1	3		11.75			2	1	1		30.75
12	16.4		12		14.00		11	1	14.08				1	7	4	9.25			1	10		11.91			4	6	2		30.83
13a-b	22.6		10	2	14.17		14		14.00				6	7	1	8.64			2	12		11.86			9	1	4		30.64
13c-14	27.2		24		14.00		24		14.00				11	11	1	8.57		1	8	12	1	11.59		2	7	5	10		30.96
15	33.6		5		14.00		5		14.00				1	4		8.80		1	1	3		11.40			2	1	2		31.00
16	36.8		12		14.00		13		14.00					12	1	9.08			1	10		11.91			4	6	3		30.92
17	39.0		5		14.00		5		14.00					4	1	9.20			3	2		11.40					5		32.00
18	46.2	2	20	2	14.00	2	22		13.92	1	1	1	4	13	4	8.62	1	1	6	11	2	11.52	1	4	11	4	4		30.25
19-20	56.6		13		14.00		12	1	14.08				6	6	1	8.62			1	8		11.89			9	3	1		30.38
21	61.4	4	15		13.79	7	10	2	13.74				7	11	2	8.75			5	13		11.72		3	6	10	1		30.45
22	67.1		15		14.00	1	14		13.93				7	6	2	8.67			4	8	1	11.77		1	6	4	3	1	30.80
23-26	96.0	3	19		13.86	2	19	1	13.95			2	2	16	2	8.82			9	10	1	11.60	3	3	9	5	1		29.90
27-29	146.7	2	5		13.71	1	6		13.86				3	4		8.57			4	3		11.43		1	2	3	1		30.57
ii) excluding fish with vertebral fusions:																													
4-6	5.0		13		14.00		13		14.00				1	8	4	9.23			3	9		11.75			5	6	2		30.77
8-9	8.7		6		14.00		6		14.00					5		9.00			1	3		11.75			4		1		30.40
10-11	12.0		4		14.00		3	1	14.25				1	2	1	9.00			1	3		11.75			2	1	1		30.75
12	16.4		11		14.00		10	1	14.09				1	6	4	9.27			1	9		11.90			4	5	2		30.82
13a-b	22.7		9	1	14.10		12		14.00				6	5	1	8.58			1	11		11.92			7	1	4		30.75
13c-14	27.5		16		14.00		16		14.00				6	8	1	8.67			3	11		11.79		1	7	3	5		30.75
15	33.6		3		14.00		3		14.00				1	2		8.67			1	2		11.67			2		1		30.67
16	36.8		12		14.00		13		14.00					12	1	9.08			1	10		11.91			4	6	3		30.92
17	38.4		4		14.00		4		14.00					3	1	9.25			2	2		11.50					4		32.00
18	45.9	1	10	1	14.00		12		14.00					8	4	9.33			3	8		11.73		1	7	2	2		30.42
19-20	56.3		7		14.00		6	1	14.14				3	3	1	8.71			1	4		11.80			5	2			30.29
21	61.0		10		14.00		8	2	14.20				3	7	1	8.82			2	7		11.78			5	6			30.55
22	66.8		10		14.00		10		14.00				3	5	2	8.90			2	6		11.75			6	2	1	1	30.70
23-26	94.4	1	17		13.94	1	16	1	14.00			1	1	14	2	8.94			6	9	1	11.69	2	2	7	5	1		30.06
27-29	148.7	1	4		13.80	1	4		13.80				1	4		8.80			2	3		11.60			2	2	1		30.80

*fish with missing values for some fin ray counts are included in calculations of mean time.

APPENDIX A (Continued...)

4. Fin ray counts in the 25P25I-BW series.

Duration of Intraparental Incubation		Right Pectoral			Left Pectoral			Dorsal					Anal							Caudal						
Nom. stage	Mean time (h)*	13	14	Mean	13	14	Mean	7	8	9	10	Mean	9	10	11	12	13	14	Mean	27	28	29	30	31	32	Mean
i) including fish with vertebral fusions:																										
2-8	7.8		4	14.00		4	14.00			4		9.00			1	3			11.75				3	1		30.25
9-10	10.5	2	8	13.80	2	8	13.80		4	4	2	8.80			6	4			11.40			3	7			29.70
11	11.7		3	14.00		3	14.00		1	1	1	9.00			2	1			11.33		1		2			29.33
12a	15.0		8	14.00		8	14.00		2	4	2	9.00		1	4	2			11.14	1	1		4	1	1	29.75
12b	19.7		5	14.00		5	14.00			4	1	9.20			3	2			11.40			3	2			29.40
13a	22.4		7	14.00	2	5	13.71		3	3	1	8.71			2	5			11.71				4	2	1	30.57
13d-14	29.0		12	14.00		12	14.00		2	10	1	8.92			8	4			11.33				9	4		30.31
15-16	33.3	2	11	13.85	2	11	13.85		8	6		8.43			4	8			11.67			2	8	4		30.14
17-18	44.6	1	11	13.92		12	14.00	2	1	10		8.62	1		8	4			11.15			1	9	3		30.15
19-20a	53.3		6	14.00	1	5	13.83	1	2	3		8.33		2	2	2			11.00			1	4		2	30.43
20b	57.3		6	14.00		6	14.00		2	4		8.67			1	4	1		12.00			2	3	1		29.83
21	61.4	1	3	13.75		3	14.00		4			8.00				4			12.00			1	3			29.75
22a	66.8	2	2	13.50	1	3	13.75	1		3		8.50			1	2		1	12.25			2	2			29.50
ii) excluding fish with vertebral fusions:																										
2-8	7.8		4	14.00		4	14.00			4		9.00			1	3			11.75				3	1		30.25
9-10	10.5	1	8	13.89	1	8	13.89		3	4	2	8.89			6	3			11.33			3	6			29.67
11	11.7		3	14.00		3	14.00		1	1	1	9.00			2	1			11.33		1		2			29.33
12a	15.2		7	14.00		7	14.00		2	3	2	9.00		1	3	2			11.17	1			4	1	1	30.00
12b	19.7		5	14.00		5	14.00			4	1	9.20			3	2			11.40			3	2			29.40
13a	22.2		5	14.00		5	14.00		2	2	1	8.80			1	4			11.80				4	1		30.20
13d-14	29.0		12	14.00		12	14.00		2	10	1	8.92			8	4			11.33				9	4		30.31
15-16	33.8	1	11	13.92	1	11	13.92		8	5		8.38			4	7			11.64			2	7	4		30.15
17-18	44.5	1	7	13.88		8	14.00		1	7		8.88			4	4			11.50			1	6	1		30.00
19-20a	53.2		1	14.00		1	14.00			1		9.00			1				11.00						1	32.00
20b	57.4		4	14.00		4	14.00			4		9.00			1	3			11.75			1	2	1		30.00
21	64.4		1	14.00		1	14.00		1			8.00				1			12.00			1				29.00
22a	66.0	2		13.00	1	1	13.50			2		9.00				1		1	13.00			1	1			29.50

*fish with missing values for some fin ray counts are included in calculations of mean time.

Appendix B. Meristic counts of fish with and without vertebral fusions in the 25P25I series. Significance levels for differences in means and variances of counts of fish with and without fusions (P_x and P_s, respectively) are based on t-tests and Bartlett's test for homogeneity, respectively. To avoid spurious differences due to differences in mean intraparental incubation times, only eggs laid at or after stage 17 are used in comparisons of those counts most significantly related to intraparental incubation time (denoted by asterisks after P_x).

Meristic series	Freshwater Incubation								Brackish Water Incubation							
	Fish Without Fusions			Fish With Fusions					Fish Without Fusions			Fish With Fusions				
	N	Mean	S.D.	N	Mean	S.D.	P_s	P_x	N	Mean	S.D.	N	Mean	S.D.	P_s	P_x
Abdominal arches	151	12.17	0.37	57	12.28	0.68	<.001	.23	117	12.06	0.24	30	12.37	0.56	<.001	.006
Caudal arches	151	21.82	0.50	57	21.53	1.04	<.001	.04	35	21.69	0.58	22	21.23	1.57	<.001	.20*
Total arches	151	33.99	0.54	57	33.81	1.25	<.001	.30	35	33.71	0.62	22	33.55	1.37	<.001	.59*
Abdominal centra	151	12.04	0.36	57	11.39	1.32	<.001	<.001	117	12.02	0.23	30	11.45	1.83	<.001	.10
Caudal centra	68	21.49	0.68	38	18.37	2.28	<.001	<.001*	117	21.34	0.56	30	18.15	3.24	<.001	<.001
Total centra	68	33.51	0.76	38	29.97	2.20	<.001	<.001*	118	33.36	0.57	30	29.60	4.36	<.001	<.001
Total vertebral ct.	68	33.80	0.59	38	33.37	1.11	<.001	.03*	35	33.50	0.54	22	33.25	1.49	<.001	.46*
Right pectoral rays	66	13.97	0.25	38	13.82	0.46	<.001	.06*	16	13.81	0.40	16	13.94	0.25	.07	.30*
Left pectoral rays	149	14.03	0.23	57	13.81	0.40	<.001	<.001	73	13.96	0.20	20	13.75	0.40	<.001	.05
Dorsal rays	148	8.95	0.61	57	8.42	0.78	.02	<.001	75	8.84	0.59	22	8.23	0.75	.14	<.001
Anal rays	132	11.75	0.45	52	11.54	0.73	<.001	.06	16	11.75	0.78	17	11.29	0.98	.36	.15*
Caudal rays	66	30.50	0.95	38	30.32	1.16	.15	.38*	75	30.00	0.79	23	30.09	0.90	.40	.66

Appendix C. Frequency (%) of fin ray abnormalities in major experimental series.

Treatment	Right Pectoral				Left Pectoral				Dorsal						Anal				Caudal				
	Type 1	Type 2	Total	N	Type 1	Type 2	Total	N	Type 1	Type 2	Type 3	Type 4	Total	N	Type 1	Type 2	Total	N	Type 1	Type 2	Type 3	Total	N
25P25I-FW	0	2.4	2.4	206	0	0.5	0.5	209	1.9	1.0	0.5	1.0	4.4	207	0	0	0	186	0.5	1.0	0.5	2.0	208
25P25I-BW	0	1.0	1.0	97	0	1.1	1.1	95	0	0	1.0	0	1.0	103	0	2.0	2.0	96	1.0	0	0	1.0	103
25P25I-FWD	2.6	0	2.6	38	0	0	0	38	0	0	2.6	0	2.6	38	0	0	0	38	0	0	0	0	39
25P30I	6.0	0.6	6.6	352	2.3	0.9	3.2	349	1.4	3.1	0.3	0.6	5.4	355	0.3	0.6	0.9	340	1.1	2.0	1.8	4.9	351
30P25I	2.6	0	2.6	76	0	0	0	78	1.3	0	0	0	1.3	79	0	0	0	78	0	0	0	0	78
30P30I	0	2.0	2.0	50	0	2.0	2.0	49	0	0	2.0	0	2.0	50	0	0	0	47	0	0	0	0	50
Total	2.9	1.1	4.0	819	1.0	0.7	1.7	818	1.2	1.6	0.6	0.5	3.9	832	0.1	0.6	0.7	785	0.7	1.1	0.9	2.7	829

APPENDIX D. Regression analyses of the influence of parental reproductive history on meristic counts.

1. Simple linear regression equations for meristic series (Y) significantly related to parental reproductive history (X). See Table 7 in text for significance levels.

Sample		Y	Equation	r^2
25P25I-FW	All fish	average pectoral rays	$\hat{Y} = 13.96 + 0.00080X$	0.067
		left pectoral rays	$\hat{Y} = 13.95 + 0.00131X$	0.111
		dorsal rays	$\hat{Y} = 8.77 + 0.00311X$	0.082
		anal rays	$\hat{Y} = 11.61 + 0.00266X$	0.106
	Fish with 25C GPTH	caudal arches	$\hat{Y} = 21.65 + 0.00185X$	0.049
		caudal centra	$\hat{Y} = 21.30 + 0.00230X$	0.051
		total centra	$\hat{Y} = 33.33 + 0.00222X$	0.042
25P25I-BW	Fish with 30C GPTH	caudal arches	$\hat{Y} = 21.61 + 0.00702X$	0.138
		total arches	$\hat{Y} = 33.63 + 0.00664X$	0.111
		total vertebral counts	$\hat{Y} = 33.44 + 0.00525X$	0.077

APPENDIX D. (Continued)

2. Polynomial regressions between selected[1] meristic series (Y) and parental reproductive history (X) for fish of 25C grandparentage in the 25P25I-FW series.

Meristic series	Goodness of Fit[2]						Regression Coefficients				
	P(0.1)	P(1.2)	P(2.3)	MSR_1	MSR_2	MSR_3	Degree	b_0	b_1	b_2	b_3
Caudal arches	.05	>.10	-	0.264	0.265		1	21.65	0.00185		
							2	21.62	0.00364	-0.00001	
Caudal centra	.025-.05	>.10	>.10	0.388	0.381	0.381	1	21.30	0.00230		
							2	21.23	0.00685	-0.00003	
Total centra	.05-.10	.10	>.10	0.447	0.439	0.444	1	33.33	0.00222		
							2	33.26	0.00696	-0.00003	
Dorsal rays	<.005	.05-.10	>.10	0.338	0.326	0.328	1	8.83	0.00337		
							2	8.75	0.00847	-0.00003	
Anal rays	<.005	.025-.05	>.10	0.198	0.186	0.182	1	11.57	0.00300		
							2	11.50	0.00785	-0.00003	
Left pectoral rays	<.005	<.005	<.005	0.027	0.020	0.033	1	13.94	0.00138		
							2	13.99	-0.00185	0.00002	
							3	13.95	0.00786	-0.00013	<0.00001

[1]Only meristic series having a significant (or nearly so) linear relationship with parental reproductive history were selected.

[2]P(n.n+1) is the probability that a polynomial of degree n+1 provides a better fit to the data than a polynomial of degree n. MSR_n is the residual mean square for a polynomial of degree n.

APPENDIX D (Continued)

3. Multiple linear regressions between meristic counts (Y) and duration of intraparental incubation (X_1) and parental reproductive history (X_2).

A. Significance levels and signs of partial regression coefficients.

Meristic series	Fish with 25C GPTH in the 25P25I-FW series		Fish with 30C GPTH in the 25P25I-BW series	
	b_1	b_2	b_1	b_2
Abdominal arches	0.75 (+)	0.11 (-)	1.00	1.00
Caudal arches	0.10 (-)	0.06 (+)	0.02 (-)	0.01 (+)
Total arches	0.18 (-)	0.48 (+)	0.02 (-)	0.01 (+)
Abdominal centra	0.71 (+)	0.82 (-)	0.27 (+)	0.55 (+)
Caudal centra	0.005 (-)	0.04 (+)	0.37 (-)	0.27 (+)
Total centra	0.02 (-)	0.09 (+)	0.53 (-)	0.20 (+)
Total vertebral counts	0.03 (-)	0.34 (+)	0.09 (-)	0.05 (+)
Average pectoral rays	>0.20 (-)	<0.01 (+)		

B. Regression equations for significant relationships.

Sample	Y	Equation	r^2
FW series	caudal arches	$\hat{Y} = 21.76 - 0.00232X_1 + 0.00177X_2$	0.071
25C GPTH	caudal centra	$\hat{Y} = 21.54 - 0.00452X_1 + 0.00214X_2$	0.129
	total centra	$\hat{Y} = 33.58 - 0.00409X_1 + 0.00197X_2$	0.089
	total vertebral counts	$\hat{Y} = 33.85 - 0.00309X_1 + 0.00085X_2$	0.066
	average pectoral rays	$\hat{Y} = 13.99 - 0.00046X_1 + 0.00080X_2$	0.116
BW series	caudal arches	$\hat{Y} = 22.04 - 0.00891X_1 + 0.00627X_2$	0.239
30C GPTH	total arches	$\hat{Y} = 34.04 - 0.00891X_1 + 0.00627X_2$	0.239
	Total vertebral counts	$\hat{Y} = 33.75 - 0.00670X_1 + 0.00488X_2$	0.148

APPENDIX E. Source of the effect of parental reproductive history on meristic counts.

The influence of parental reproductive history on meristic counts may result from differences between the eggs or sperm of new and of experienced parents (i.e., a "prefertilization" effect) or from environmental differences experienced by developing embryos within parents with short or long reproductive histories. Only counts produced by eggs fertilized artificially could distinguish between a prefertilization influence and a very early postfertilization environmental effect. A prefertilization effect can however be distinguished from a more typical environmental influence (i.e., one which operates during a prolonged period of lability) by comparing fish with long and short PRH within groups with long or short intraparental incubation times (Table E.1). If the effect of PRH is due to gametic differences established before fertilization, such comparisons should reveal similar differences regardless of intraparental incubation time. If the effect is due to environmental influences, these comparisons should be interpreted in terms of transfers between intra- and extraparental levels of some environmental factor. Given the latter possibility and most possible response patterns, a change in the direction of meristic differences between fish with long and short PRH is expected between comparisons at short and long intraparental incubation times (if the long intraparental incubation times are near or exceed times of meristic fixation).

Differences between numbers of dorsal or anal fin rays of fish with short and long PRH are similar regardless of intraparental incubation time (Table E.1). This result is consistent with a prefertilization effect but, because of the relatively late fixation times of these meristic series, it does not rule out the possibility of an effect of PRH due to incubation environments. Vertebral differences attributable to PRH are significant only among fish with short intraparental incubation but are in the same direction regardless of intraparental incubation time (Table E.1). Since fish with long intraparental incubation in these comparisons were, on the average, laid at about the fixation time of vertebral number (about 80 h after fertilization at 25C), the latter result suggests a prefertilization effect of PRH.

The incongruity between the meristic series strongly affected by PRH and those affected by intraparental incubation time also suggests a prefertilization influence of PRH rather than an environmental effect operating during prolonged periods of lability. (For example, dorsal ray counts are greatly affected by PRH but apparently unaffected by intraparental incubation time.)

In summary, the observed association between meristic counts and PRH can be interpreted as arising from prefertilization influences; an alternate interpretation, attributing the association to early postfertilization environmental influences, could be valid only if there were a very brief period of lability in response to such influences,

quite unlike the prolonged periods of lability already known to occur in this and other species in response to temperature, light and probably salinity.

TABLE E.1. Mean meristic counts of fish with long and short parental reproductive histories within groups with long or short intraparental incubation times.

Meristic series	Short Intraparental Incubation			Long Intraparental Incubation		
	PRH ≤5 days (N=10 or 11)	PRH>60 days (N=14 or 15)	Signif-icance	PRH ≤ 5 days (N=17, 18 or 19)	PRH >60days (N=13 or 15)	Signif-icance
Caudal arches	21.64	22.00	.01	21.53	21.80	.15
Total arches	33.73	34.00	.03	33.74	33.93	.40
Caudal centra	21.36	21.87	.006	21.21	21.40	.39
Total centra	33.45	33.80	.07	33.26	33.53	.27
Total vertebral counts	33.59	33.93	.03	33.58	33.73	.44
Dorsal rays	8.73	9.27	.02	8.72	9.27	.02
Anal rays	11.50	11.93	.04	11.41	12.00	.002
Mean intraparental incubation time (h)	17.3	10.0		82.8	90.3	

*anal rays
†dorsal rays

APPENDIX F. Mean meristic counts of fish incubated in fresh- or brackish water (FW or BW, respectively). Fish are grouped by developmental time at transfer to freshwater or at collection if incubated in brackish water. Significance levels (p) of differences between means are according to t-tests.

1. Entire sample. Note that confounding with PRH should tend to obscure salinity effects.

	Developmental Time (h) at Transfer to Freshwater or at Collection											
	All times			<37.5			37.5-57.9			58.0-190.0		
Meristic series	FW	BW	P	FW	BW	P	FW	BW	P	FW	BW	P
Vertebrae N=	151	117-118		38	45		55	34-35		58	37	
Abdominal arches	12.17	12.06	.005	12.16	12.04	.10	12.16	12.09	.32	12.17	12.05	.06
Caudal arches	21.82	21.74	.21	21.82	21.76	.51	21.91	21.85	.62	21.74	21.62	.30
Total arches	33.99	33.81	.007	33.97	33.80	.06	34.07	33.94	.29	33.91	33.68	.07
Abdominal centra	12.04	12.02	.53	12.05	12.00	.28	12.02	12.03	.88	12.05	12.03	.70
Caudal centra	21.53	21.34	.009	21.55	21.31	.03	21.65	21.41	.07	21.40	21.32	.57
Total centra	33.57	33.36	.007	33.61	33.31	.007	33.67	33.46	.13	33.45	33.35	.51
Total vert. cts.	33.84	33.61	<.001	33.84	33.58	.003	33.95	33.73	.08	33.74	33.53	.08
Fin Rays N=	132-149	71-75		34-38	38-39		49-55	14-17		49-57	18-19	
Right pectoral	13.99	13.93	.07	14.00	13.97	.33	14.00	14.00	1.00	13.98	13.79	.07
Left pectoral	14.03	13.96	.03	14.05	13.97	.08	14.02	13.94	.23	14.02	13.94	.35
Dorsal	8.95	8.84	.19	9.08	8.95	.37	8.85	8.76	.58	8.96	8.68	.08
Anal	11.75	11.52	.006	11.79	11.37	<.001	11.78	11.57	.13	11.69	11.79	.54
Caudal	30.65	30.00	<.001	30.81	29.90	<.001	30.82	29.94	<.001	30.38	30.26	.63
Mean PRH (days)	38.4	54.8*		63.2	82.6*		23.7	31.8*		35.6	41.4*	
Mean Intraparental Incubation (h)	45.3	32.8		12.2	17.7		33.0	35.6		78.4	51.4	

*Mean PRH of fish with countable fin rays are much greater than values shown (most fish with uncountable fin rays had PRH ≤5 days).

APPENDIX F (Continued)

2. Subsample with 25C GPTH and PRH >30 days. Note significant differences in dorsal ray counts.

	Developmental Time (h) at Transfer to Freshwater or at Collection								
	All times			<37.5			≥37.5		
Meristic series	FW	BW	P	FW	BW	P	FW	BW	P
Vertebrae N=	36	34-35		15	26		21	8-9	
Abdominal arches	12.11	12.06	.44	12.13	12.08	.57	12.10	12.00	.38
Caudal arches	21.83	21.76	.52	21.87	21.81	.64	21.81	21.62	.40
Total arches	33.94	33.83	.33	34.00	33.88	.31	33.90	33.67	.36
Abdominal centra	12.06	12.00	.43	12.07	12.00	.46	12.05	12.00	.73
Caudal centra	21.61	21.35	.04	21.73	21.38	.05	21.52	21.25	.20
Total centra	33.67	33.37	.02	33.80	33.38	.01	33.57	33.33	.30
Total vert. cts.	33.83	33.63	.07	33.93	33.67	.01	33.76	33.50	.25
Fin Rays N=	33-36	30-34		14-15	24-25		19-21	6-9	
Right pectoral	14.03	13.97	.16	14.00	13.96	.45	14.05	14.00	.56
Left pectoral	14.06	13.94	.05	14.07	13.96	.15	14.05	13.86	.11
Dorsal	9.25	8.79	.002	9.33	8.92	.04	9.19	8.44	.003
Anal	11.97	11.37	<.001	12.00	11.29	<.001	11.95	11.67	.18
Caudal	30.81	30.09	.001	30.47	30.00	.12	31.05	30.33	.05
Mean PRH (days)	107.2	118.2		115.3	110.4		101.4	140.9	
Mean Intraparental Incubation (h)	44.3	20.4		7.0	16.3		70.9	32.2	

APPENDIX G. Mean meristic counts of fish incubated in light or darkness. Fish incubated in darkness are grouped by developmental time at transfer to darkness. Significance levels of differences between counts of fish reared in light or transferred to darkness are based on t-tests and shown in parentheses. Except for caudal rays (which are not affected by PRH), only counts of fish with PRH greater than 30 days are shown. (In the entire sample, confounding with PRH tended to obscure most meristic differences but may have contributed to the increased vertebral numbers among fish transferred to darkness at intermediate stages.) Counts are shown both for subsamples of mixed and uniform GPTH.

Variable	GPTH	Sustained incubation in light	Developmental Time (h) at Transfer to Darkness <36.8	36.9-46.0	46.1-72.0
i) All PRH					
Sample size	All	119	9	10	17
Caudal rays	All	30.72	30.67(.85)	30.80(.78)	30.12(.008)
Mean PRH (days)	All	33.6	143.1	72.5	78.9
Mean intraparental incubation time (h)	All	31.7	11.1	23.6	36.1
ii) PRH >30 days					
Sample size	All	33-35	7	9-10	15-16
	25C	21-23	7	-	10-11
	Unknown	8	-	6-7	-
Abdominal arches	All	12.09	12.00(.43)	12.20(.32)	12.06(.78)
	25C	12.09	12.00(.44)	-	12.00(.33)
	Unknown	12.12	-	12.29(.47)	-
Caudal arches	All	21.86	21.43(.01)	21.90(.73)	21.94(.61)
	25C	21.87	21.43(.015)	-	22.00(.53)
	Unknown	21.88	-	22.00(.37)	-
Total arches	All	33.94	33.43(.002)	34.10(.42)	34.00(.69)
	25C	33.96	33.43(.04)	-	34.00(.83)
	Unknown	34.00	-	34.29(.30)	-
Abdominal centra	All	12.00	11.86(.20)	12.10(.29)	11.94(.40)
	25C	12.00	11.86(.31)	-	11.91(.42)
	Unknown	12.00	-	12.14(.30)	-
Caudal centra	All	21.71	21.29(.03)	21.90(.24)	21.75(.80)
	25C	21.74	21.29(.03)	-	21.73(.94)
	Unknown	21.62	-	22.00(.08)	-
Total centra	All	33.71	33.14(.004)	34.00(.09)	33.69(.85)
	25C	33.74	33.14(.004)	-	33.64(.55)
	Unknown	33.62	-	34.14(.05)	-
Total vertebral cts.	All	33.87	33.36(.001)	34.10(.25)	33.91(.75)
	25C	33.89	33.36(.025)	-	33.86(.87)
	Unknown	33.88	-	34.29(.11)	-
Average pectoral rays*	All	14.03	13.93(.08)	13.94(.09)	13.81(.03)
	25C	14.05	13.93(.10)	-	13.86(.01)
	Unknown	14.00	-	14.00(1.00)	-
Dorsal rays	All	9.09	8.71(.19)	8.80(.20)	8.80(.15)
	25C	9.30	8.71(.03)	-	8.80(.03)
	Unknown	8.88	-	9.00(.62)	-
Anal rays	All	11.91	11.86(.67)	11.60(.10)	11.40(.008)
	25C	11.95	11.86(.54)	-	11.40(.03)
	Unknown	11.75	-	11.71(.89)	-
Mean PRH (days)	All	85.3	160.7	72.5	82.2
	25C	101.9	160.7	-	85.0
	Unknown	47.2	-	76.0	-
Mean intraparental incubation time(h)	All	19.0	10.5	23.6	36.2
	25C	14.2	10.5	-	41.7
	Unknown	31.1	-	18.8	-

*Averaged on each fish. Right and left pectoral ray counts responded similarly to transfers to darkness (although responses were usually greater in the right fin).

APPENDIX H. Mean meristic counts of fish collected during different sampling periods. All fish compared have 25C GPTH. Significance levels are according to t-tests.

Variable	Sampling Period: October 1976 - March 1977	Sampling Period: May - October 1977	Significance
N=	41-44	42-43*	
Abdominal arches	12.23	12.16	.45
Caudal arches	21.73	21.70	.80
Total arches	33.95	33.86	.42
Abdominal centra	12.07	12.05	.81
Caudal centra	21.48	21.30	.19
Total centra	33.55	33.35	.18
Total vertebral counts	33.83	33.66	.14
Right pectoral rays	14.05	13.98	.08
Left pectoral rays	14.02	14.07	.41
Dorsal rays	9.10	8.98	.37
Anal rays	11.74	11.67	.53
Caudal rays	30.43	30.71	.17
Mean PRH (days)	43.6	41.0	
Mean intraparental incubation time(h)	44.3	59.6	

*N=33 for anal rays

APPENDIX I. Tests for post-hatching thermolability of meristic counts. Significance levels are according to t-tests. Results are shown only for the entire sample but are similar in the subsample of 25C GPTH.

	Fish transferred from 25C to 30C after hatching		Fish kept at 25C after hatching		
	Mean	S.D.	Mean	S.D.	Significance
Vertebrae N=	11		117-118		
Abdominal arches	12.09	0.30	12.06	0.24	.69
Caudal arches	21.64	0.50	21.74	0.49	.49
Total arches	33.73	0.65	33.81	0.54	.66
Abdominal centra	12.09	0.30	12.02	0.23	.32
Caudal centra	21.27	0.47	21.34	0.56	.69
Total centra	33.36	0.50	33.36	0.56	1.00
Total vertebral counts	33.55	0.52	33.61	0.52	.71
Fin Rays N=	10-11		74-80		
Right pectoral	14.00	0.00	13.92	0.27	.34
Left pectoral	13.91	0.30	13.95	0.23	.62
Dorsal	8.91	0.30	8.85	0.60	.60
Anal	11.70	0.68	11.54	0.60	.44
Caudal	29.82	0.98	29.98	0.81	.56
Mean PRH (days)	41		54		
Mean intraparental incubation time(h)	47		33		

APPENDIX J. Mean meristic counts and intraparental incubation times in the temperature break experiments.

1. Mean meristic counts in the entire 25P30I and 25P25I-FW samples and in subsamples with reduced confounding with PRH and GPTH. Caudal ray counts are not shown since they are not affected by PRH and GPTH. Counts are not shown for the 30P25I and 30P30I samples since both contained only fish with 25C GPTH and (with one exception in each sample) PRH greater than 5 days.

Sample	Mean developmental time (h) at temperature transfer (if applicable)	Total Vertebral Counts				Average Pectoral Rays				Dorsal Rays				Anal Rays				Sample Sizes			
			Excluding fish with PRH ≤5 days				Excluding fish with PRH ≤5 days				Excluding fish with PRH ≤ 5 days				Excluding fish with PRH ≤5 days				Excluding fish with PRH ≤5 days		
		Entire Sample	Excluding fish with unknown PRH	Including fish with unknown PRH all GPTH	Including fish with unknown PRH 25C GPTH	Entire Sample	Excluding fish with unknown PRH	Including fish with unknown PRH all GPTH	Including fish with unknown PRH 25 GPTH	Entire Sample	Excluding fish with unknown PRH	Including fish with unknown PRH all GPTH	Including fish with unknown PRH 25C GPTH	Entire Sample	Excluding fish with unknown PRH	Including fish with unknown PRH all GPTH	Including fish with unknown PRH 25C GPTH	Entire Sample	Excluding fish with unknown PRH	Including fish with unknown PRH all GPTH	Including fish with unknown PRH 25C GPTH
25P30I	5.3	33.42	33.38	33.42	33.55	13.82	13.84	13.82	13.78	9.22	9.24	9.22	9.20	11.88	11.88	11.88	11.89	18	17	18	10
	10.7	33.11	33.11	33.11	33.17	13.70	13.88	13.70	13.63	9.16	9.22	9.16	9.17	11.93	11.94	11.93	11.95	31	18	31	24
	19.3	33.07	33.00	33.07	33.10	13.55	13.70	13.55	13.45	9.30	9.20	9.30	9.30	11.91	11.80	11.91	12.00	28	10	28	21
	22.4	33.08	33.08	33.08	33.18	13.72	13.72	13.72	13.55	9.11	9.11	9.11	9.00	11.72	11.72	11.72	11.91	18	18	18	11
	27.4	32.95	32.95	32.95	32.93	13.55	13.55	13.55	13.54	9.05	9.05	9.05	9.07	11.52	11.50	11.52	11.36	21	20	21	14
	32.0	33.10	33.06	33.10	33.10	13.58	13.56	13.58	13.57	9.00	9.00	9.00	9.00	11.65	11.61	11.65	11.73	20	18	20	15
	36.6	32.98	32.98	32.98	32.94	13.52	13.50	13.52	13.53	9.04	9.04	9.04	9.06	11.64	11.65	11.67	11.75	29	27	28	17
	46.0	33.27	33.29	33.31	33.26	13.53	13.58	13.55	13.52	9.03	9.08	9.06	9.00	11.73	11.77	11.75	11.70	33	26	32	23
	51.8	33.21	33.21	33.21	33.16	13.40	13.40	13.40	13.41	9.00	9.00	9.00	9.00	11.67	11.67	11.67	11.69	21	21	21	16
	62.5	33.53	33.53	33.56	33.44	13.19	13.23	13.20	13.29	8.82	8.86	8.81	8.75	11.65	11.64	11.69	11.75	18	15	17	8
	72.4	33.42	33.46	33.45	33.50	13.58	13.65	13.62	13.66	8.79	8.88	8.85	8.91	11.89	11.88	11.88	11.95	30	27	28	24
	77.0	33.76	33.92	33.93	33.93	13.67	13.80	13.75	13.75	8.71	8.83	8.79	8.79	11.67	11.80	11.75	11.75	17	12	14	14
	107.2	33.50	33.62	33.62	33.33	13.64	13.62	13.62	13.67	8.86	9.00	9.00	9.00	11.14	11.50	11.50	11.67	7	4	4	3
	132.0	33.72	33.71	33.71	33.83	13.75	13.75	13.75	13.80	8.78	8.86	8.86	9.00	11.89	11.71	11.71	11.67	9	7	7	6
	138.0	33.73	33.73	33.73	33.73	14.05	14.05	14.05	14.05	9.00	9.00	9.00	9.00	11.91	11.91	11.91	11.91	11	11	11	11
	269.7	34.00	34.03	34.03	34.03	13.86	13.84	13.85	13.84	8.94	8.94	8.94	8.94	11.82	11.87	11.81	11.87	18	16	17	16
25P25I-FW	-	33.84	33.85	33.88	33.84	14.01	14.03	14.04	14.06	8.95	9.03	9.04	9.20	11.75	11.89	11.90	11.89	151	80	103	61

APPENDIX J. Continued...

2. Mean intraparental incubation times in lots transferred between temperatures during development. Values shown are for the entire samples; similar values occurred in subsamples omitting fish with PRH of 5 days or less. Values for sustained temperature controls are as follows: 25C - 45.3 h; 30C, conceived shortly after parental temperature transfer - 27.1 h; 30C, conceived long after parental temperature transfer - 45.2 h.

Transfers from 25C to 30C		Transfers from 30C to 25C	
Mean Developmental Time at Temperature Transfer (h)	Mean Intraparental Incubation Time (h)	Mean Developmental Time at Temperature Transfer (h)	Mean Intraparental Incubation Time (h)
5.3	2.7	4.8	1.9
10.7	5.0	7.6	3.3
19.3	9.1	9.6	4.3
22.4	11.7	11.1	5.0
27.4	18.5	18.6	8.8
32.0	18.4	29.5	14.2
36.8	20.4	46.2	26.2
46.0	28.4	64.1	50.4
51.8	29.0	74.2	55.4
62.8	47.9	77.0	59.0
72.4	43.4	126.5	109.1
77.0	35.0	171.0	153.8
106.3	62.0		
132.0	89.1		
138.0	117.0		

APPENDIX J. Continued...

3. Mean arch and centrum counts in the 25P30I and 25P25I-FW series (in subsamples omitting fish with PRH of 5 days or less).

Mean Developmental Time at Temperature Transfer(h)	Arches			Centra		
	Abdominal	Caudal	Total	Abdominal	Caudal	Total
5.3	12.00	21.61	33.61	11.91	21.19	33.06
10.7	12.03	21.23	33.26	11.94	20.94	32.87
19.3	12.00	21.11	33.11	11.89	21.04	32.93
22.4	12.00	21.17	33.17	12.00	21.00	33.00
27.4	12.00	21.00	33.00	12.00	20.90	32.90
32.0	12.05	21.10	33.15	12.05	21.00	33.05
36.6	12.00	21.07	33.07	11.75	20.89	32.64
46.0	12.12	21.25	33.38	11.75	21.12	32.88
51.8	12.05	21.33	33.38	11.86	21.00	32.86
62.5	12.29	21.53	33.82	11.94	21.00	32.94
72.4	12.07	21.54	33.61	11.88	21.20	33.07
77.0	12.21	21.93	34.14	12.00	21.50	33.50
107.2	12.50	21.50	34.00	12.25	20.75	33.00
132.0	12.14	21.71	33.86	11.86	21.43	33.29
138.0	12.09	22.00	34.09	12.09	21.27	33.36
269.7	12.06	22.06	34.12	12.06	21.88	33.94
25C control	12.17	21.84	34.02	12.06	21.56	33.62