絶食ペヘレイ仔魚の生残に及ぼす水温の影響

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著者	Struessmann, C.A. 隆島, 史夫
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Effects of Temperature upon Survival and Histological Changes of Starved Pejerrey *Odontesthes bonariensis* Larvae*1

Carlos Augusto Strüssmann*2 and Fumio Takashima*2

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Preliminary results on the effects of incubation and rearing temperatures on histological and morphometrical features and on starvation induced mortality of first feeding pejerrey larvae are presented. Newly hatched larvae at 17.5°C were nearly 8% larger than those at 21.5°C, had significantly larger lower jaw lengths and mouth gapes but smaller yolk reserves owing to the longer incubation periods. The differences decreased with growth, but still favored larvae at lower temperatures. Larvae hatched at 19.5°C had intermediate length and availalbe energy, but relatively larger oil globules. Energy consumption and growth rates were directly proportional to temperature at both pre and post hatching periods. Yolk conversion efficiencies were initially similar and later maximal at 19.5°C. Yolk and oil were never completely absorbed in starved and dying larvae. Mortality of larvae starved throughout amounted to 50% at 5.5, 7.1 and 7.3 days respectively at 21.5, 19.5 and 17.5°C, but total mortality varied little from an average of 9 days irrespective of temperature. Survival in the delayed feeding trials decreased with longer starvation periods at 21.5 and 19.5°C. Such a trend could not be clearly identified at 17.5°C until total mortality. Histological criteria developed in an earlier study were employed to analyze the dynamics of changes and loss of tissue integrity and their correlation with survival expectancy. Initial changes were slower at 19.5°C, but leveled at days 3 and 4. After 4 and 5 days of starvation, the percentages of larvae bearing similar pancreatic and intestinal degeneration were similar, but liver seemed to be least affected at 17.5°C. It becomes evident that temperature interacts with starvation to affect potential survival and that utilization of histological methods to assess starvation induced mortalities should take temperature into consideration.

In a previous study, histological degeneration of the pancreas, liver and posterior intestine of starved pejerrey *Odontesthes bonariensis* larvae were investigated.[1] Daily histological changes, thought to constitute reliable indicators of starving condition,[1-3] were accompanyed by decreasing survival potential, though a clear relationship between them could not be established. However, it might be of interest to understand how nutritional condition (as evidenced by histological features) correlates with the ability to feed and survive after a period of starvation and the role of environmental factors. Braum[4] pointed out that temperature is the most important external factor acting upon developing fish embryos and larvae, determining morphological features, timing of ontogenetic events and even the behavior of newly hatched larvae. Likewise, the size of larvae at the onset of feeding and the time to irreversible starvation are also largely determined by water temperature.[5] Although studies substantiating these assumptions are numerous, the matter seems far from settled. For instance, Howell[6] found hardly any effect of temperature on the overall efficiency of yolk utillization and maximum size of yellowtail flounder larvae, and concluded that it will exert little influence on their survival potential. Of more predictable nature seems to be the time to onset of irreversible starvation, which has been shown to decrease with increasing temperatures for a number of species.[7-11]

Incubation of pejerrey eggs within a certain range of temperatures (15–25°C) does not affect perceivably either hatching rates or the percentage of viable larvae,[12] and similar tolerance has been observed for other Atherinid species.[13,14] However, within this range, effects of temperature upon efficiency of yolk utilization (California grunion),[14] size of larvae (grunion and Atlantic silverside),[14,15] and time to starvation death (topsmelt and grunion)[13] have been reported. Point-of-no-return (PNR)[7,8] has been observed at 3.5 days after hatch-

*1 Basic Studies on the Culture of Pejerrey-II.

*2 Laboratory of Fish Culture, Tokyo University of Fisheries, Konan, Minato, Tokyo 108, Japan (Carlos A. Strüssman, 隆島史夫: 東京水産大学).

ing (50% threshold) or 5 days (no survivors) when pejerrey larvae were incubated at fluctuating temperatures (20–21°C) and reared at 21.5°C.[1] On the contrary, May[16] found first feeding grunion larvae extremely tolerant to starvation.

In the present study, we have attempted to investigate the effects of incubation temperature on morphometrical, histological and energetic aspects, and to discuss on how these factors interact with starvation to affect potential survival. Results of preliminary experiments and information of relevance to the manangement of natural and cultured populations of pejerrey are also presented.

Materials and Methods

Pejerrey eggs from a single 3-year-old female were inseminated at the Kanagawa Prefectural Freshwater Fish Experimental Station, and brought to the Laboratory of Fish Culture, Tokyo University of Fisheries, after hardening of the chorion. Temperature was maintained at 20.5°C during transportation. Within 10 hrs of fertilization, eggs were divided into 3 groups and gradually acclimated in conical, flowing water flasks to 3 temperature regimes: 17.5, 19.5 and 21.5°C (±0.3°C) for incubation.

Fourteen 700 μm meshed net cages of 0.2 *l* were each stocked with 50 larvae hatched around the time of 50% hatching of each temperature level. These cages were kept in a large container where no food was provided. Beginning 24 hrs after the time of 50% hatching, a new group was gently transferred to another identical but otherwise "fed" container every day. Cladocerans (predominantly *Moina* sp. but also some *Daphnia* sp.) were sieved in a 500 μm mesh to remove larger individuals and introduced in excess to the "fed" container 3–4 times daily. Flowing water and weak aeration ensured uniform distribution of plankton through the larval enclosures. Larvae in six groups never received food. Mortality in all groups was monitored in 12 h intervals. All surviving larvae in the delayed-feeding groups were counted, measured and weighed by day 15.

Samples for histological observation were taken from continuously starved groups following the protocols of the previous study.[1] Likewise, the daily histological changes of starved pejerrey larvae reported by Strüssmann and Takashima,[1] to whose descriptions and figures the reader is refered, were used as diagnostic standards to assess the effect of temperature upon the process of tissue degeneration. The histological appearances of liver, pancreas and posterior intestines of larvae at hatching and at 1, 2, 3, 4 and 5 days in that study were considered here as degeneration grades 0, 1, 2, 3, 4 and 5 respectively. In this way, higher grades indicate more profound degeneration. In addition, yolk sac and oil globule outlines in all serial sections of three individuals per group were drawn in camera lucida. Surfaces were calculated with a computerized planimeter and multiplied by the thickness of the section to obtain yolk and oil volumes. Energy available at the start of embryonic development was assessed by direct calorimetry. After dechorionation, the contents of 250 eggs fixed in formalin were rinsed in distilled water, freeze dried and combusted in a bomb calorimeter.

Data on total length and wet weight were obtained from anesthetized specimens. These were later fixed in 4% $CaCO_3$ buffered formalin. Groups of newly hatched larvae and those starved for 2 and 4 days were stained for skeletal structures according to the methods of Potthoff.[17] Specimens were then mounted on glycerin-jelly[18] and the length of the lower jaw was measured under a microscope.

Results

Morphometry

Incubation times, weight and length at the time of 50% hatching are summarized in Table 1. Hatching was delayed at lower temperatures, but newly hatched larvae were significantly larger in length. On the other hand, weight at hatching varied in direct proportion to temperature. Due to an accident, newly hatched larvae at 19.5°C were erroneously weighed and therefore are not presented. Changes in total length of starved larvae are depicted in Fig. 1. Length of larvae reared at 19.5 and 21.5°C increased significantly after hatching, while growth at 17.5°C was negligible. Since growth on yolk reserves was largely restricted to the inital 24 h, groups of larvae fasted for 1, 2, 3, and 4 days were pooled to obtain an average maximum length at each temperature. After 2 days, differences in length were reduced, but still significantly different between lower (17.5 and 19.5°C) and higher (21.5°C) temperatures (Table 1). Lower jaw length at hatching, and consequently mouth gapes, followed similar trends as total length. Later, shrinkage was evident at 17.5°C and 21.5°C.

Table 1. Size and morphology of larvae incubated and reared at three constant temperatures

Average temperature (°C)	Incubation period *1 (h)	Weight at hatching (mg)	Length at hatching (mm)	Maximum length *2 (mm)	Mandible length at hatching (mm×10)	Mandible length at 2 and 4 days *3 (mm×10)	Mouth gape (90°) at hatching *4 (mm×10)	Mouth gape (45°) at hatching *4 (mm×10)
		1.42 a	7.08 a	7.41 b	4.20 ab	4.13 a	5.70 a	4.67 a
21.5	240	.03	.04	.03	.04	.02	.05	.04
		(17)	(17)	(41)	(10)	(20)	(10)	(10)
			7.36 b	7.61 c	4.27 bc	4.28 bc	5.80 a	4.76 a
19.5	288	—	.03	.02	.07	.03	.10	.08
			(20)	(40)	(10)	(19)	(10)	(10)
		1.29 b	7.62 c	7.63 c	4.56 d	4.33 c	6.19 b	5.07 b
17.5	384	.04	.03	.03	.02	.03	.02	.02
		(20)	(20)	(39)	(10)	(20)	(10)	(10)

All measures as mean, SE and sample number (within brackets). Groups with the same letter are not significantly different ($P>0.05$) in the Duncan's New Multiple Range Test.

*1 Time elapsed from fertilization to 50% hatching.

*2 Length increments are result of growth based solely on endogenous food sources. Data from four groups of approximately 10 individuals each were pooled.

*3 Groups starved for 2 and 4 days were pooled to obtain an average mouth size during early development.

*4 Mouth gape = mandible length $\times \sqrt{2-\cos\infty}$, after Shirota.[19]

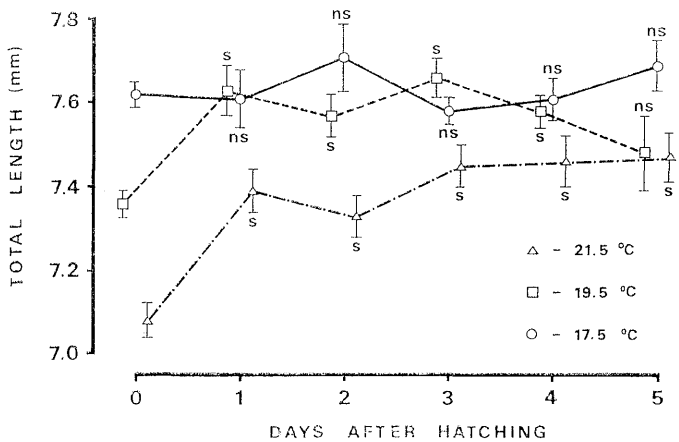

Fig. 1. Changes in total length (mean±SE) of starved larvae after hatching. Mean daily values are compared to length at hatching within each temperature level with Duncan's New Multiple Range Test and significant differences ($P<0.05$) are indicated (S).

Yolk Utilization

The volumes of yolk and oil globules were converted to available energy under the following assumptions. Yolk fraction at hatching contained 60% water and 10% bound lipid as dry matter;[20] densities of lipid and protein were assumed as 0.920 g/m*l* (20°C)[21] and 1.379 g/m*l*[22] respectively. Calorific estimates of 9.441 cal/g for oil and 5.404 cal/g for yolk[23] were employed. Daily changes in available energy from yolk sac (total = yolk fraction + oil globule) and of the oil globule are represented in Fig. 2; equations used to develop the curves of best fit are listed in Table 2. At hatching, total energy as well as energy from the yolk fraction (obtained by subtraction) were maximal at 21.5°C, while energy from oil globule peaked at 19.5°C. Initial differences were greatly reduced by day 2 and virtually disappeared after 3 days of starvation. Both oil and yolk were retained in minimal amounts until death by starvation. However, while oil absorption seemed to cease after a few days at all temperatures, yolk absorption ceased only at 17.5°C.

An average of 1.970 cal per egg was available at the onset of embryonic development. This value, total energies from Fig. 2 and data on larval length from Table 1 were used to estimate the efficiency of yolk utilization during incubation and rearing to maximum length. The efficiency of yolk utilization or growth efficiency as termed

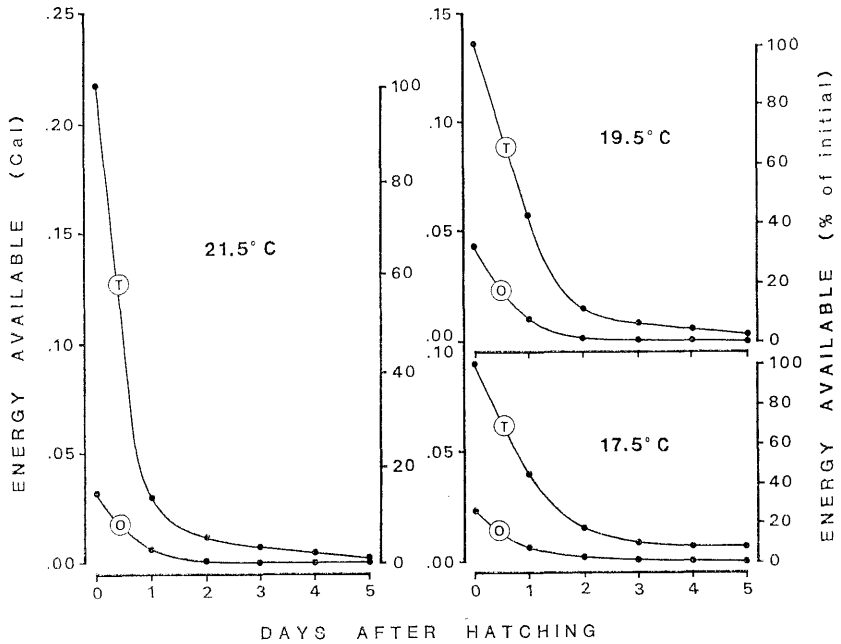

Fig. 2. Changes in Absolute (left) and Percentual (right) energy available from yolk sac (Ⓣ, total) and oil globule only (Ⓞ) at different temperatures after hatching. Equations are listed in Table 2. Dots represent regressed daily values to facilitate comparison.

Table 2. Regression of energy available (Y, cal, total or from oil globule) on time after hatching (X, days) of starved larvae held at different temperatures.

Temperature °C	Energy source	Equation	n	r
21.5	total	$Y=0.2172-0.3863X+0.2816X^2-0.0989X^3+0.0166X^4-0.0011X^5$	18	0.9690
	oil	$Y=0.0319-0.0406X+0.0190X^2-0.0038X^3+0.0003X^4$	18	0.9832
19.5	total	$Y=0.1358-0.0729X-0.0256X^2+0.0268X^3-0.0065X^4+0.0005X^5$	17	0.9926
	oil	$Y=0.0436-0.0494X+0.0210X^2-0.0039X^3+0.0003X^4$	17	0.9226
17.5	total	$Y=0.0898-0.0603X+0.0086X^2+0.0033X^3-0.0011X^4+0.0001X^5$	18	0.9930
	oil	$Y=0.0235-0.0325X+0.0212X^2-0.0075X^3+0.0013X^4-0.0001X^5$	18	0.9464

Table 3. Growth rate, energy consumption, and growth efficiency during incubation and rearing to maximum length on yolk reserves at different temperatures

Temperature °C	Incubation				Rearing to maximum length			
	Time (days)	Growth Rate (mm/day)	Energy Consumption (cal/day)	Growth Efficiency (mm/cal)	Time (days)	Growth Rate (mm/day)	Energy Consumption (cal/day)	Growth Efficiency (mm/cal)
21.5	10	0.7082	0.1753	4.040	1	0.3250	0.1881	1.728
19.5	12	0.6133	0.1528	4.014	1	0.2500	0.0778	3.213
17.5	16	0.4762	0.1175	4.053	1	0.0110	0.0493	0.223

by Ryland and Nichols[24] can be computed by the formula growth efficiency (mm/cal) = growth rate (mm/day)/energy consumption (cal/day). It can be seen in Table 3 that growth rate and energy consumption were directly proportional to temperature at both pre and post hatching periods. Growth efficiency, however, was remarkably constant during incubation but lower at extreme temperatures after hatching.

Survival

Survival in the fasted groups decreased faster at 21.5°C in comparison to lower temperatures (Fig. 3). Fifty percent mortality at 21.5, 19.5 and 17.5°C was attained at 5.6, 7.1, and 7.3 days respectively. On the contrary, little difference from an average of 9 days was observed from hatching to 100% starvation death in all groups. After feeding with cladocerans, survival in the delayed feeding trials was lower than obtained previously by feeding rotifers to the larvae.[1] For this reason, results are presented as relative survival rates, the maximum obtained was arbitrarily considered as 100% (Fig. 4d). Survival decreased proportional-

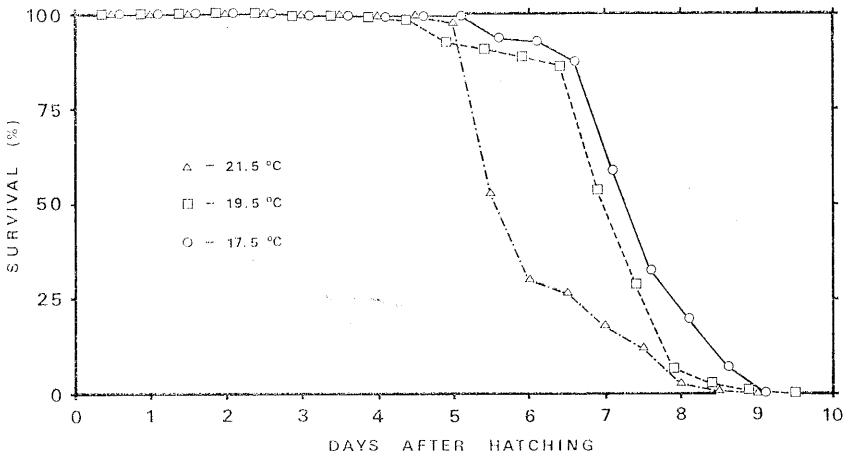

Fig. 3. Survival of larvae starved throughout at different temperatures. Each value represents the mean of at least three groups.

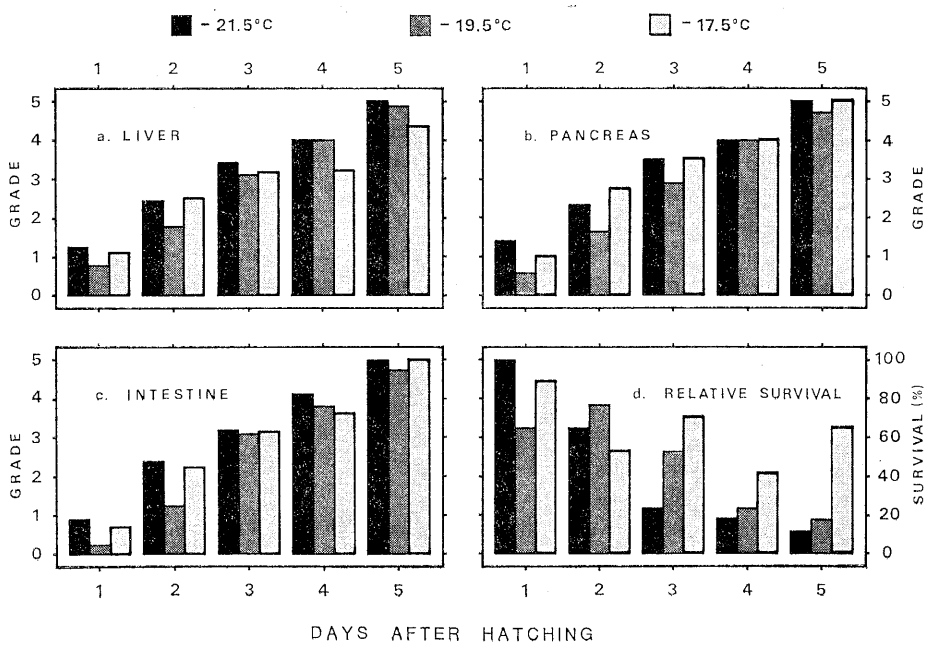

Fig. 4. Average daily histological grades and relative survival of pejerrey larvae. Histological grades of liver (a), pancreas (b), and intestine (c) of larvae starved at different temperatures. See Materials and Methods and Strüssmann and Takashima[1] for definition of the grades. Survival after 15 days (Y, %) when first feeding was delayed for various times (X, days) at different temperatures is shown in Fig. 4d.

Table 4. Test of homogeneity on the proportions of larvae with similar histological appearance at different temperatures. Nine to 11 larvae were examined daily for each temperature level. Letters by the χ^2 values indicate (a) homogeneity of (b) heterogeneity

Tissue	Days of starvation	Histological grade	Observed frequency 21.5°C	19.5°C	17.5°C	Expected frequency	Degrees of freedom	P at .05	χ^2
Pancreas	3	2	—	22.22	—	8.00	4	9.488	6.262_a
		3	50.00	66.67	50.00	56.00			
		4	50.00	11.11	50.00	36.00			
	4	3	—	—	—	—	—	—	—$_a$
		4	100.00	100.00	100.00	100.00			
		5	—	—	—	—			
	5	4	—	28.57	—	11.76	2	5.991	2.041_a
		5	100.00	71.43	100.00	88.24			
Liver	3	2	—	—	—	—	2	5.991	2.400_a
		3	60.00	88.89	83.33	76.00			
		4	40.00	11.11	16.67	24.00			
	4	3	—	—	80.00	21.05	2	5.991	14.189_b
		4	100.00	100.00	20.00	78.95			
		5	—	—	—	—			
	5	4	—	14.29	66.67	18.75	2	5.991	5.993_b
		5	100.00	85.71	33.33	81.25			
Posterior Intestine	3	2	—	11.11	—	4.00	4	9.448	2.000_a
		3	80.00	66.67	83.33	76.00			
		4	20.00	22.22	16.67	20.00			
	4	3	—	20.00	40.00	15.79	4	9.448	4.784_a
		4	88.89	80.00	60.00	78.95			
		5	11.11	—	—	5.26			
	5	4	—	28.57	—	13.33	2	5.991	2.041_a
		5	100.00	71.43	100.00	86.67			

ly with longer periods of fasting before first feeding at 21.5 and 19.5°C. Survival was rather variable at 17.5°C and a definite trend could not be ascertained. However, it never dropped below 40% until 5 days after hatching, after which no larvae was able to survive. A single larvae survived at 19.5°C when food was first presented by day 6.

Histological Changes

Temperature of incubation was found to exert little, if any, effect upon the degree of histological differentiation at hatching. Furthermore, temperature specific regressive processes were not observed after the onset of tissue depletion. Sections of liver, pancreas and posterior intestine of starved larvae from all temperatures were given a grade corresponding to the standard they most resembled (see Materials and Methods). Average daily degeneration grades are presented in Figs. 4a, b and c. As a general trend, loss of integrity was slower at 19.5°C, though initial differences greatly decreased after 3 days. Liver glycogen (liver, grade 1), intestinal supranuclear bodies (intestine, grade 0), and pancreatic acinar arrangement (pancreas, grade 0) were conserved longer at the intermediate temperature. Because survival in the groups starved for long periods ($\geqslant$3 days) could be related to larvae bearing less pronounced histological degeneration, a test of homogeneity[25] was run (Table 4). After 4 and 5 days of starvation, the percentages of larvae bearing similar pancreatic and intestinal degeneration were similar, but liver seemed to be less affected at 17.5°C (Figs. 4a, b, c, Table 4).

Discussion

Based on these results, it became evident that incubation temperature may affect the chances of survival of pejerrey larvae after hatching. Even though growth efficiency during incubation was seen to be rather constant, the temperature during early embryonic development determines size, morphology and the amount of endogenous nutriments remaining at the time of hatching.

Length at hatching was found to be inversely

related to incubation temperature as observed in *Menidia menidia*.[15] However, due to the fact that experimental conditions in both studies do not span the full range of viable temperatures,[12,13] the linearity of the relationship can not be ascertained.

Ehrlich and Muszynski[14] investigated growth, behavior and energetics of California grunion larvae shifted to a range of temperatures after hatching. Growth efficiency and maximum size were maximal at temperatures between 18 and 23°C. Though a sudden change in temperature coinciding with hatching might mimic natural events in the early life of grunion embryos (see May[16]), it might not be typical of most Atherinid species. Hence, the latter will likely encounter thermal conditions similar to those prevailing during incubation. As has been demonstrated for other species, post hatching growth efficiencies are dependent upon quantity and composition of yolk reserves and metabolic expenditure, which in turn depend on temperature, size and activity of both embryos and larvae.[14,20,26,27] An interplay of these factors might have caused early differences in size of yellowtail flounder[6] and chum salmon[27] larvae to be smoothed off by the end of yolk-sac period, and a mechanism of compensation between higher metabolic expenditures and higher yolk conversion rates has been suggested.[6] Likewise, such a compensation seems evident between the two lower temperatures investigated in the present experiment as shown in Tables 1, 2, 3 and Figs. 1, 2, while increased energy consumption at the highest is striking. However, the extent to which nutritional differences on yolk reserves (Fig. 2) might have affected the growth rates after hatching, especially at 21.5°C, is unknown.

Besides the implications of size on foraging and predator aviodance abilities[5] and on growth rates,[15] larger body and mouth sizes (Table 1), coupled to lower metabolic demands of larvae at the lower temperatures might be equally advantageous when food is scarce or not readily available. At 17.5°C, most pejerrey larvae will be able to feed and survive even if first feeding is delayed for as many as 5 days (Fig. 4d). On the other hand, larvae must find suitable food within 2 days at 21.5°C if the same survival levels are to be obtained. Furthermore, most larvae at 21.5°C were found reluctant to strike on prey during the first day, further reducing the time available for feeding commencement. Inasmuch as these larvae possessed well developed and functional digestive tracts, the reluctancy to feed is not yet understood. It might be related to larger yolk reserves and possible mechanical compression of the anterior portion of the intestine as observed in larval seabass.[28] Indeed, histological examination of newly hatched pejerrey larvae revealed narrower gut lumens at 21.5°C than their counterparts at lower temperatures.

Intermediate temperatures might strike a balance between 17.5 and 21.5°C in terms of activity, size, metabolic demands and potential survival. This assumption is supportaed by the results of growth efficiency, energy consumption, histological degeneration and survival of larvae kept at 19.5°C in the present experiment. Furthermore, it is suggested that pejerrey larvae incubated at high and shifted to low temperatures at hatching migh exhibit prolonged survival due to their greater yolk reserves and smaller size. This hypothesis seems to hold true for laboratory reared Clupeoid larvae as pointed out by Hempel and Blaxter.[8] Moreover, it might be a natural phenomenon in the early life of Atherinid larvae should the thermoregulatory behavior of grunion larvae[14] constitute a common feature of this group.

The differences in time to starvation death and response to delayed feeding (Fig. 3, 4d) as compared to a previous report[1] might be greatly attributed to changes in container size[29] and kind of food offered respectively.[30] Nevertheless, the longest delay that any larvae could withstand were approximated in both experiments (4 days against 5–6 days). Of greater importance is the fact that the interaction of temperature with starvation was more easily detected by delayed feeding experiments than by measuring time to starvation death alone. A higher ecological significance of the former has been suggested by Blaxter and Hempel.[7]

Histological analysis provided further evidence that the effects of higher temperatures were compensated by larger yolk reserves and smaller size. Degeneration rates were not as affected as would be expected under different thermal regimes (Fig. 3a, b, c). Likewise, the persistence of health indicators, viz. hepatic glycogen, intestinal supranuclear bodies and healthy pancreatic structure (lower histological grades), consists indirect indication that the onset of energy deficit and consequent tissue resorption were delayed at 19.5°C, but nevertheless proceeded at similar rates afterwards.

In an earlier study, Strüssmann and Takashima[1] could not find reliable indicators among degenerative processes to relate to decreasing survival potential. In a similar manner, the present ex-

periment demonstrates that a single factor, viz. temperature, sufficed to induce different survival rates despite similar histological appearances (compare the trends on Fig. 3a, b, c with d). Moreover, the correlation of tissue degeneration on potential survival apparently gets weaker at lower temperatures. Nevertheless, further research is needed to dissociate larvae with lower degrees of degeneration from those survivors within a group, as well as to investigate the relative importance of hepatic histological integrity on survival potential. It can be concluded that utilization of histological criteria alone, although useful in assessing starving condition, might not provide conclusive insight on the survival potential of first feeding pejerrey larvae. Unless the relative importance of biotic and abiotic factors interacting with starvation are properly evaluated, perhaps in a form of a model, irrefutable data and reliable predictions might be by no means obtainable.

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