



Morphological development and allometric growth patterns in hatchery-reared California halibut larvae

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Morphological development, allometric growth and behaviour of hatchery-reared California halibut *Paralichthys californicus* were studied from hatching to metamorphosis (42 days post hatch, dph) at 18°C. Mean standard length (L_S) of larvae and juveniles increased from 2.1 mm at hatching to 10.5 mm at metamorphosis with the increase in length being approximately linear. Stages of morphological development were described using the alphabetic staging (A–I) used for other flatfish species. Organogenesis and differentiation were more rapid and complex in yolk-sac (hatching, stage A–3 dph, stage B), preflexion (3–19 dph, stages B–C), and flexion larvae (from 20 to 23 dph, stages D–E), as larvae developed most of their sensory, feeding, respiratory and swimming systems. After notochord flexion at 24–25 dph (stage F), most morphological changes were related to the progressive transformation from a bilateral symmetrical larva to an asymmetrical benthic juvenile (42 dph, stages G–I).

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INTRODUCTION

Morphogenesis and differentiation are rapid and complex processes during early ontogeny of fishes, when newly hatched larvae undergo dramatic changes in their body shape, morphology, metabolism, swimming abilities and behaviour as they transform into a juvenile or adult form, usually over a relatively short time period (Osse & van den Boogart, 1995; van Snik *et al.*, 1997; Gisbert, 1999; Koumoundouros *et al.*, 1999). The change in body shape leads to the formation of characteristic morphologies and allometric growth patterns. Under unfavourable developmental conditions, the changes can also lead to structural defects affecting the growth and survival of young fishes.

Information about the morphological development and growth patterns of young fishes is important for fisheries management and aquaculture (Chatain, 1994; Koumoundouros *et al.*, 1994, 1999; Bengtson, 1999). Recognition of normal patterns and detection of developmental defects can be used to improve larval rearing techniques through modification of environmental parameters and

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feeding practices. Such an approach can also be used to compare specimens from different egg batches. Furthermore, it allows the estimation of the quality of juveniles and their suitability for stocking or further rearing. As the most widely used markers, length and mass, show no strict relationship to development (Segner *et al.*, 1995), morphological and functional markers should be more useful indicators for the development of optimal rearing techniques.

Californian halibut *Paralichthys californicus* (Ayres), a pleuronectid, is a highly valued fish species along the California coast extending from Washington State to Baja California. This species supports important commercial and recreational fisheries (Gadomski *et al.*, 1990) and is also a promising candidate for marine aquaculture. The early development of California halibut was first described by Oda (1991) from specimens collected from the near-shore zone of southern California. The study, however, only provided characters to separate this species from other flatfishes occurring in this area, rather than a detailed sequence of morphological changes during early life stages.

The objectives of the present study were to (1) describe the morphological development and allometric growth patterns and (2) present a comprehensive staging series for hatchery-reared California halibut from hatching to metamorphosis.

MATERIAL AND METHODS

Larvae used in the present study were obtained from a natural spawning of domesticated broodstock of California halibut held at Redondo Beach, California. Eggs were collected in a rectangular tank and transferred to a cylindrical incubation tank (60 l). Water temperature during incubation was 18–20°C and gentle aeration was provided using an air diffuser. Hatching began 36–48 h after spawning and 8000 1 day-old larvae were shipped by air at a density of 1000 larvae l⁻¹ to the University of California Davis (Biological and Agricultural Engineering Department Laboratory), where they were acclimated and divided into three cylindrical static rearing tanks (52 l) at a density of 50 larvae l⁻¹.

Larvae were fed for the first time at 3 days post hatch (dph) with rotifers *Brachionus plicatilis* fed with yeast and enriched for 12 h with Rotimac[™] (Bio-Marine, Inc). Initially, prey concentration was 5 rotifers ml⁻¹ and was progressively increased to 15 rotifers ml⁻¹ at 20 dph. From 17 to 42 dph, fish were fed with enriched *Artemia* nauplii (Bio-Marine Algamac 3050[™]), while weaning onto pellets started at 27 dph (Bio-Marine Artemac #2[™]). Rearing tanks were cleaned once a day by siphoning the bottom to remove waste and dead larvae. Water temperature (Omega HH82 digital thermometer), dissolved oxygen (DO) (YSI model 58) and salinity (YSI model 33) were measured daily throughout the rearing period. Water temperature, DO and salinity were 18.2 ± 0.2°C, 7.8 ± 0.1 mg l⁻¹ and 30.5 ± 0.5‰, respectively. Fish were exposed to 12L : 12D photoperiod using overhead fluorescent lights.

Twenty specimens were sampled daily at random from rearing tanks, anaesthetized with tricaine methanesulphonate (MS 222), preserved in 10% formalin and stored in the dark at 6–8°C, for later examination of the morphology of sensory, feeding and swimming structures. The external development of eyes, mouth, nares, gills, fins and the absorption of yolk sac were observed under ×10, 16, 25 and 50 magnification of a dissection microscope (Leica Wild TYP181300). Stages of morphological development were described using the alphabetic staging system developed by Fukuhara (1986) for *Paralichthys olivaceus* Temminck & Schlegel and adapted to other flatfish species (Keefe & Able, 1993; van Maaren & Daniels, 2000). From each sampled specimen, seven morphometric characteristics associated with swimming abilities, vision and feeding were measured to the nearest 0.01 mm using a stereoscopic microscope with a camera lucida

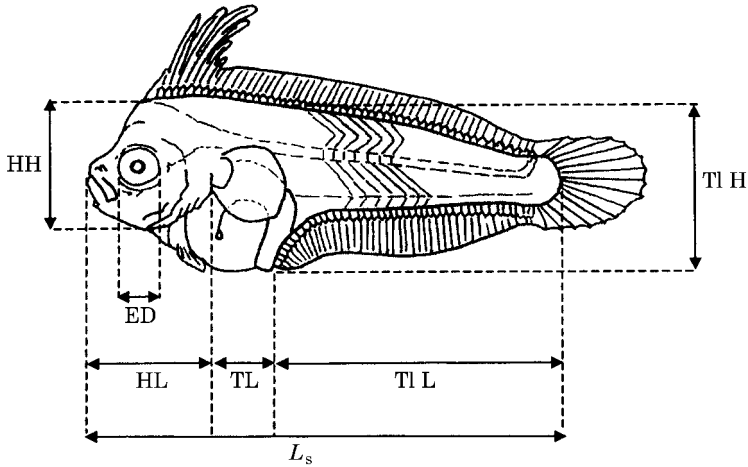


FIG. 1. Morphometric characters measured in California halibut from hatching to metamorphosis (42 dph). ED, eye diameter; HH head height; HL, head length; L_s , standard length; TIH, tail height; TIL, tail length; TL, trunk length.

and a digital pad (Micro-Plan II, Laboratory Computer Systems, Inc.). These characteristics were: (1) standard length (L_s); (2) the distance between the anus and the tip of the notochord (TIL, tail length); (3) the distance between the anterior base of the anal fin and the base of the dorsal fin (TIH, tail height); (4) the distance between the operculum and the anterior tip of the anal fin (TL, trunk length); (5) the distance from the anterior tip of the head to the operculum (HL, head length); (6) the head height measured at the level of the operculum (HH); (7) the eye diameter (ED) (Fig. 1). In yolk sac larvae, the diameter (maximum and minimum) of the ellipsoidal yolk sac was also measured and the volume (mm^3) calculated using the following formula: $V=0.1667 \pi LH^2$; where H is the minimum diameter and L is the maximum diameter of the yolk sac mass (Heming & Buddington, 1988). All measurements were taken along lines parallel or perpendicular to the horizontal axis of the body (Gisbert, 1999). Abnormal specimens were excluded from the study.

Allometric growth was calculated as a power function of L_s using non-transformed data: $y=a L_s^b$; where y was the measured character, a the intercept, and b the growth coefficient (Fuiman, 1983). When isometric growth occurred, $b=1$; allometric growth was positive when b was >1 , and negative when <1 . The equations were established from regressions performed on log-transformed data, using L_s as the independent variable (Gisbert, 1999). Inflexion points of growth curves were determined according to van Snik *et al.* (1997). The x - y data set was sorted according to increasing L_s . Regression lines were calculated for $L_{s \min}$ until $L_{s \text{ intermediate}}$, and for $L_{s \text{ intermediate}}$ until $L_{s \max}$, where $L_{s \text{ intermediate}}$ varied iteratively from $L_{s \min} + 2$ to $L_{s \max} - 2$. Also, t tests were done to check whether the growth coefficients for $L_{s \min}$ $L_{s \text{ intermediate}}$ and $L_{s \text{ intermediate}}$ $L_{s \max}$ differed significantly. The $L_{s \text{ intermediate}}$ value that resulted in the largest t was defined as the inflexion point. Growth coefficients were compared statistically by means of the t test.

No specific experiments were done to test swimming ability of larvae and only general observations were made in the rearing tanks during day-light conditions.

RESULTS

MORPHOLOGICAL DEVELOPMENT

At hatching, larvae measured 2.1 ± 0.1 mm L_s (mean \pm s.d.) and had the head bent downwards with the hatching gland on the lower head surface. The mouth

opening, eyes and gill clefts were absent (Fig. 2, stage A). Auditory capsules containing otoliths were visible in the upper posterior head region. No fins were detected with the exception of a wide primordial finfold that bordered the notochord. The finfold was wider on the dorsal part of the trunk and narrowed at the caudal portion. Larvae had a large yolk sac (mean \pm s.d. volume 0.083 ± 0.010 mm³, $n=20$) containing several oil droplets in the posterior portion that migrated towards the anterior region with further development. Pigmentation was limited to few scattered melanophores located in the dorsal posterior part of the notochord and finfold.

Between 1 and 2 dph (2.6 ± 0.1 mm L_S) nearly 80% of the initial yolk reserves were utilized and yolk sac volume decreased considerably (0.011 ± 0.010 mm³). Unpigmented eyes started to develop and lens appeared; mouth opened, and rudimentary maxilla and Meckel's cartilage were detected. The digestive tract could be observed as a straight tube under the notochord. The liver appeared ventral to the developing gut and could be differentiated as a whitish non-lobular tissue mass. The urinary bladder appeared next to the rectal duct. Pectoral fins developed but fin rays were absent. Scattered melanophores developed in the dorsal part of the head and in the abdominal region, while those from the notochord and finfold increased in size and number.

At 3 dph (2.7 ± 0.1 mm L_S) larvae had depleted their yolk sac completely (some fish still had small oil globules) and started exogenous feeding. Eyes were pigmented and mouth size increased due to the development of the maxilla and Meckel's cartilage. Rudiments of gill cover could be distinguished as a deep cleft but gill arches were not observed until 4 dph (3.0 ± 0.1 mm L_S). The number and size of melanophores increased in all body regions, but especially along the notochord (Fig. 2, stage B). Although differentiation proceeded as larvae grew, few morphological changes took place during days 5–12. As a consequence of the splanchnocranium growth and differentiation, the mouth position changed from subterminal to terminal. Some scattered melanophores were detected in the pectoral fin folds at 6 dph (3.1 ± 0.1 mm L_S). Between 5–7 dph, folding was observed in the digestive tract, while at 11 dph (4.2 ± 0.2 mm L_S) the digestive tract coiled and increased in size.

At 13 dph (4.4 ± 0.4 mm L_S), the dorsal finfold protruded slightly where the future head dorsal rays would develop (Fig. 2, stage C). At 15 dph (4.6 ± 0.4 mm L_S), three dorsal rays developed and extended out from the finfold; elongation began with the second dorsal ray and progressed with the first and third. The preanal finfold size was reduced considerably as a consequence of the development of the abdominal cavity and digestive tract. Pigmentation in the ventral abdominal cavity increased due to the development of internal and external melanophores.

Between 17–18 dph (4.9 ± 0.3 mm L_S) the mandible no longer protruded over the maxilla and both looked symmetrical. The finfold narrowed at the caudal peduncle and the first caudal fin rays ($n=7$) appeared on the ventral side of the caudal finfold tip and developed in an anterior-posterior direction (Fig. 2, stage D). At 20 dph (5.3 ± 0.4 mm L_S), some fish started to show an upward turn of the notochord tip (c. 45°). Notochord flexion was a common feature in most fish at 21 dph (5.7 ± 0.3 mm L_S). Rudiments of dorsal and ventral fin ray support appeared and larvae had five long dorsal pigmented rays on the head.

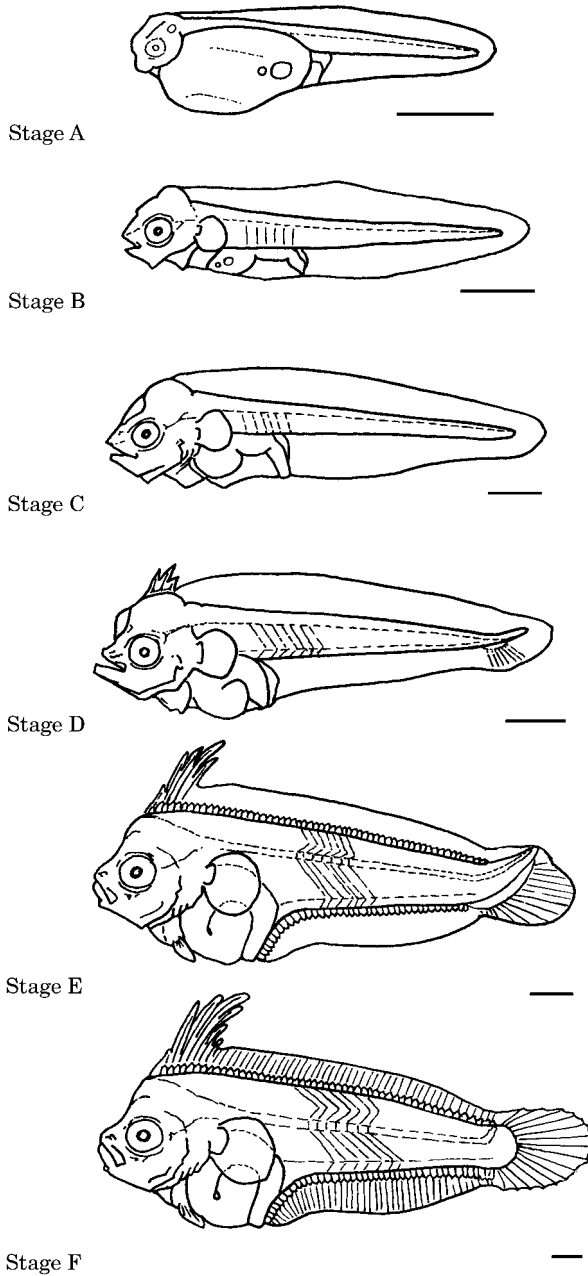


FIG. 2. Morphological development of California halibut from hatching to the flexion larvae. Stage A, newly hatched yolk-sac larva; stage B, first feeding preflexion larva (3 dph); stage C, preflexion larva at early stages of dorsal ray development (13 dph); stage D, larva at early stages of notochord flexion (18 dph); stage E, larva at intermediate stages of notochord flexion (45°) (20 dph); stage F, flexion larvae (24 dph). Melanophores were not drawn. Scale bar, 500 μ m.

Melanophores covered the abdominal region, but the digestive tract was still visible (Fig. 2, Stage E).

Between 23 (6.1 ± 0.4 mm L_S) and 25 dph (65 ± 04 mm L_S), notochord flexion was completed (90°). The finfold narrowed at the caudal peduncle and dorsal and ventral fins were separated from the caudal fin. The caudal fin had 17–19 segmented rays, while non-segmented rays could be observed in dorsal ($n=64$ – 68) and ventral ($n=48$ – 50) fins. The preanal finfold was completely reabsorbed and pelvic fins appeared, with four non-segmented rays (Fig. 2, stage F). Head rays started to be reabsorbed and decreased in size, while they did not resemble the rest of the dorsal fin rays until 38 dph (8.4 ± 0.6 mm L_S). The head started to change in shape as the eye canal developed. Between 27–28 dph (7.2 ± 0.4 mm L_S), either the right or the left eye started to migrate in a proportion of 1 : 1 (52.3 and 47.7% of right and left eyed fish, respectively, χ^2 , $P > 0.05$, $n=120$) (Fig. 3, stage G), reaching the dorsal midline at 35 dph (7.8 ± 0.6 mm L_S) (Fig. 3, stage H). Body shape changed from a bilaterally symmetrical larval to a young asymmetrical juvenile at 42 dph (10.1 ± 0.5 mm L_S) when eye migration was accomplished. At this stage, dorsal and ventral fin rays were already segmented, melanophores covered the ocular side of the body and the digestive tract was no longer visible, while the blind side remained unpigmented. Fish resembled ‘miniature adults’, but no scales were formed (Fig. 3, stage I).

ALLOMETRIC GROWTH

From hatching to metamorphosis, growth in length (L_S) was related to age (dph) by a linear function (Fig. 4). During this period, body proportions and growth rates changed considerably. Head length and height showed different growth patterns. Growth in head length could be divided into three different stages during larval development [Fig. 5(a)]. From hatching to 18–19 dph, growth was positively allometric ($b=1.28 \pm 0.03$) and from the inflexion point at 5.1 mm L_S until 8.0 mm L_S at 30 dph, it increased sharply ($b=1.89 \pm 0.05$). Head length growth became nearly isometric ($b=1.07 \pm 0.07$) between 30 and 42 dph. The head height growth could be divided in two distinct stages [Fig. 5(b)]; from hatching to day 18–19 dph, growth was positively allometric ($b=1.23 \pm 0.03$) and from the inflexion point at 5.0 mm L_S to day 42, head height increased considerably ($b=1.50 \pm 0.04$).

Eye diameter and tail length growth showed similar allometric growth patterns. The eyes grew isometrically between 1–2 dph and 22–23 dph ($b=0.96$). Eye diameter growth was positively allometric ($b=1.71$) from the inflexion point at 6.0 mm L_S [Fig. 5(c)].

Tail length growth was isometric ($b=1.00 \pm 0.02$) until 6.0 mm L_S and then increased to positively allometric ($b=1.51 \pm 0.03$) [Fig. 5(d)]. Tail height growth was ‘triphasic’ during larval development [Fig. 5(e)]. From hatching to 4.9 mm L_S at 18–19 dph, tail height growth was positively allometric ($b=1.28 \pm 0.05$), while from 4.9 to 6.7 mm L_S (24–25 dph), tail height growth rapidly increased ($b=5.0 \pm 0.02$). Thereafter, the allometric coefficient decreased ($b=1.18 \pm 0.06$). Trunk length increased negatively allometrically ($b=0.83 \pm 0.02$) from hatching to 6.7 mm L_S and then decreased in size ($b=-2.45 \pm 0.02$) [Fig. 5(f)].

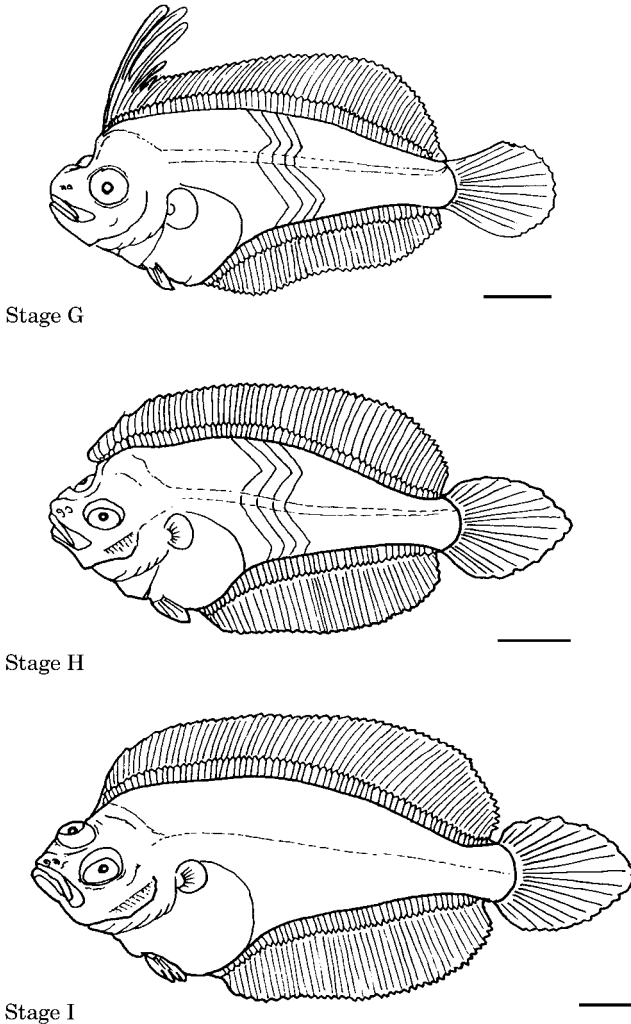


FIG. 3. Morphological development of California halibut larvae from notochord flexion to metamorphosis. Stage G, postflexion larvae (30 dph); stage H, postflexion larvae with the migrating eye reaching the dorsal midline (35 dph); stage I, early juvenile (42 dph). Melanophores were not drawn. Scale bar, 1 mm.

BEHAVIOUR

At hatching, larvae were positively buoyant and were distributed throughout the water surface. One day later, as a consequence of yolk sac resorption, larval buoyancy decreased and larvae sank in the water column. During the first 2 days, larval behaviour was characterized by periods of resting and swimming activity, while at 3 dph, most larvae actively swam and maintained their position in the water column. California halibut larvae were visual feeders: feeding behaviour was characterized by the twisting of the entire body into a sinusoidal shape when the prey was detected, followed by quick bursts towards it. At 19–21 dph, larvae changed from an anguilliform swimming style characterized by flexure of large amplitude over a large part of their body to a subcarangiform

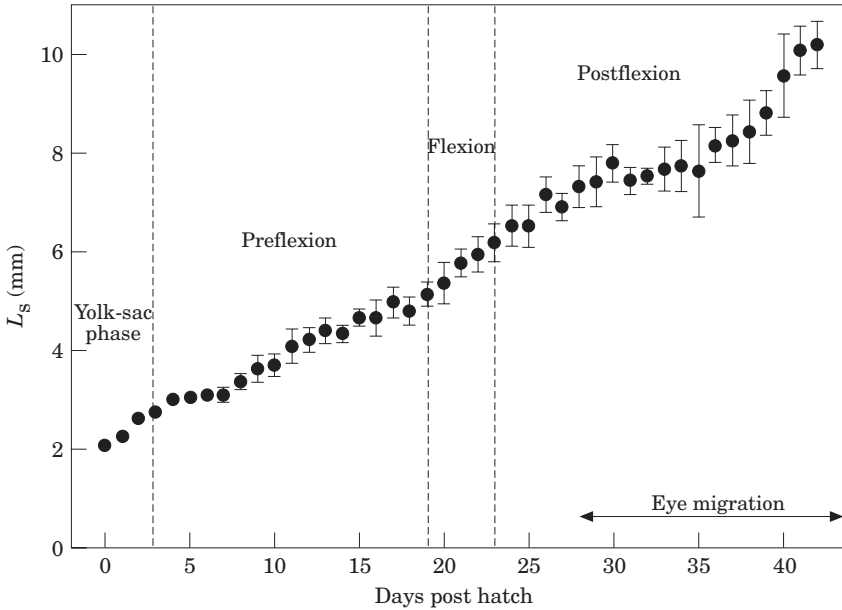


FIG. 4. Growth in standard length of California halibut from hatching to the completion of metamorphosis. During the period studied, growth could be defined by $L_S = 0.18 + 2.02$ days post hatch ($r^2 = 0.96$; $P < 0.01$; $n = 440$).

style in which only the caudal region flexed but the rest of the body remained relatively rigid. Coinciding with the completion of notochord flexion at 23–25 dph, larvae started to settle to the bottom for short intervals, though they continued to feed in the water column. As eye migration proceeded, the time larvae spent on the bottom increased, and they were completely benthic when eye migration was completed. At this stage, fish were able to feed both in the water column and on the bottom of the tank. No cannibalistic behaviour was observed up to 42 dph, but cannibalistic juveniles were detected at older ages.

DISCUSSION

As for most teleost species, functional systems of hatching California halibut larvae were still incomplete and undeveloped. Consequently, growth, development and differentiation of newly hatched larvae resulted in changes in their body shape, morphology, metabolism, swimming abilities and behaviour. This transformation into juveniles occurred in a relatively short time. The early stages of California halibut development (up to 42 dph) could be divided into different phases according to morphological development and allometric growth coefficients: the yolk sac phase, between hatching (stage A) and yolk sac resorption and first feeding at 3 dph (stage B); the preflexion larval phase (from 3 to 19 dph, stages B–C); the flexion period (from 20 to 23 dph, stages D–F); and the postflexion phase, from notochord flexion to the completion of metamorphosis in 10 mm L_S larvae at 42 dph (stages G–I). The sequence of morphological events described in the present study from hatchery-reared specimens was similar to the first morphological description of this species from field-collected material

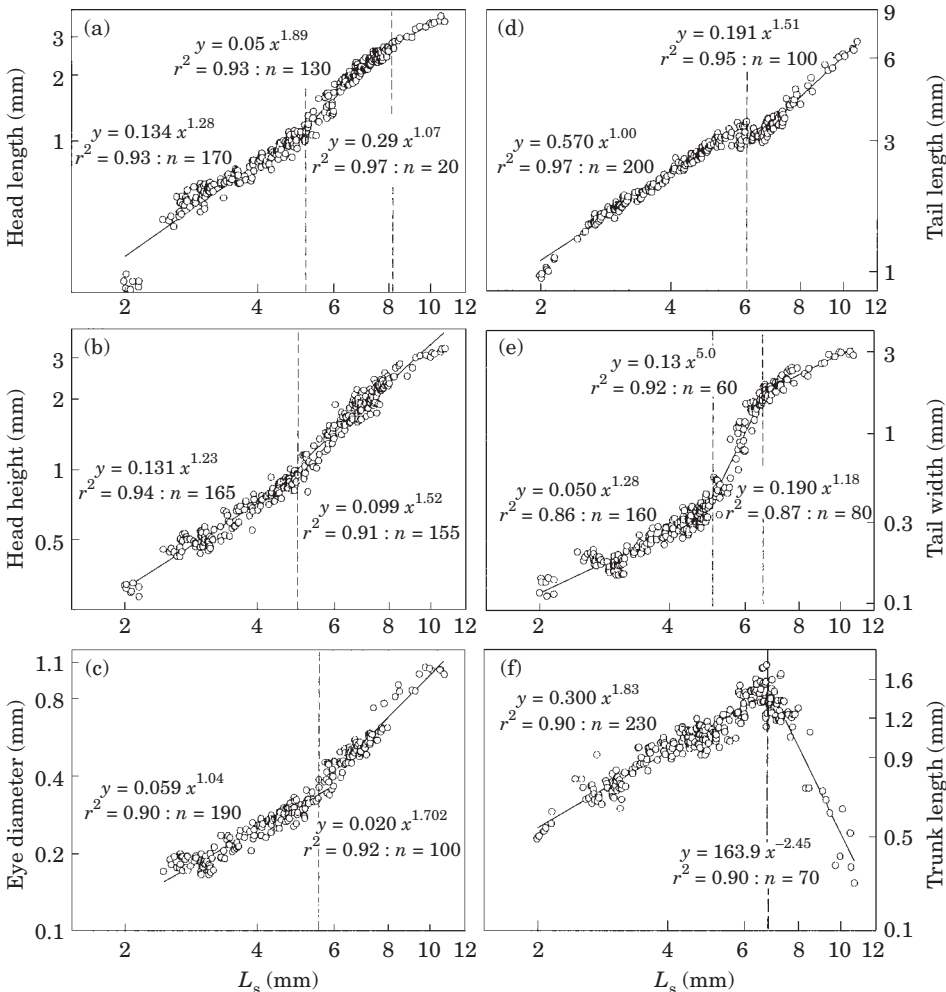


FIG. 5. Allometric growth equations and relationships of different selected body regions with standard length in California halibut during early stages of development (from hatching to metamorphosis at 42 dph). (a) Head length (first inflexion point at 5.1 mm L_s , at 17–18 dph; second inflexion point at 8.0 mm L_s , at 30 dph); (b) head height (inflexion point at 5.0 mm L_s , at 17–18 dph); (c) eye diameter (inflexion point at 6.0 mm L_s , at 22–23 dph); (d) tail length (inflexion point at 6.0 mm L_s , at 22–23 dph); (e) tail height (first inflexion point at 4.9 mm L_s , at 17–18 dph; second inflexion point at 6.7 mm L_s , at 24–25 dph); (f) trunk length (inflexion point at 6.7 mm L_s , at 24–25 dph). Note logarithmic axes.

in the near-shore zone of the South California Bight reported by Oda (1991), although some differences appeared in the time sequence at which changes took place (e.g. eye pigmentation, notochord flexion, head rays resorption, completion of metamorphosis). Similarly, in the present study, the analysis of the morphological development and allometric growth patterns of selected body regions indicated that the acquisition of the juvenile phenotype was not completed until 10 mm L_s . These results were slightly different from those reported in natural environments at temperatures ranging from 20–24° C (Allen, 1988; Gadomski & Caddell, 1991; Oda, 1991), where metamorphosis was

observed at *c.* 8–9 mm L_S , coinciding with the passive transport of larvae to coastal and estuarine areas. These differences between hatchery-reared and wild fish may be attributable to differences in environmental parameters that regulate their development (water temperature, Seikai *et al.*, 1986; photoperiod, Youson, 1988; hormonal cues Tanaka *et al.*, 1995; Solbakken *et al.*, 1999; Schreiber & Specker, 2000).

During early ontogeny of California halibut, body proportions and growth rates changed considerably. Similarly to *P. olivaceus* (Seikai *et al.*, 1986), the head length of California halibut larvae had a positive allometric biphasic growth before becoming nearly isometric at 8 mm L_S . The positive allometric growth of head length from hatching to 5.1 mm L_S (18–19 dph) was linked to the development of nervous (midbrain and hindbrain), sensory (vision, olfaction and lateral line), respiratory (gill arches and filaments), and feeding (splanchnocranium skeleton) systems of larvae. As a consequence of the differentiation of neural and sensorial structures (positive allometric growth of eyes, development of the olfactory organ and neuromasts), larvae would be able to react to light stimuli, detect zooplankton prey and potential predators in the water column, and start feeding exogenously when yolk-sac reserves were depleted. The development of gill arches and filaments would allow a shift from cutaneous to branchial respiration, resulting in a better oxygen supply and an increase in swimming activity. The development of feeding structures (functional jaw) in such a short period of time is a common feature in pelagic larvae (Pittman *et al.*, 1990; Morrison & MacDonald, 1995; Hunt von Herbing, 2001) and may improve prey capture thus increasing larval growth and survival chances (Hunt von Herbing, 2001).

The abrupt change in head proportions from the inflexion point at 5.1 mm L_S (18–19 dph) until 8.0 mm L_S at 30 dph seemed to be associated with the development of the eye canal and the onset of eye migration. The increase in head height growth detected at 5.0 mm L_S until the end of larval metamorphosis appeared to be associated with the progressive transition from a symmetrical larva into a bilaterally asymmetrical juvenile. This transition resulted in profound changes in the organization of the splanchnocranium skeleton, especially jaws and visceral arches, as feeding and respiratory structures had to be readapted to this new asymmetrical shape and benthic life (Seikai *et al.*, 1986; Francis & Turingan, 2000; Hunt von Herbing, 2001).

The present study revealed that hatchery-reared California halibut could be either left or right eyed (1 : 1), confirming the observations made by Gadomski *et al.* (1990) on wild fish. These results, however, differed from most pleuronectid species described, which can be either left-eyed, such as *Paralichthys lethostigma* Jordan & Gilbert (Powell & Henley, 1995; van Maaren & Daniels, 2000), *Paralichthys dentatus* (L.) (Keefe & Able, 1993), *P. olivaceus* (Fukuhara, 1986), *Paralichthys albigutta* Jordan & Gilbert (Powell & Henley, 1995) and *Paralichthys orbignyanus* (Valenciennes) (Figueiredo & Menezes, 2000), or right-eyed such as *Platichthys flesus* (L.) (Cooper & Chapeau, 1998) and *Pseudopleuronectes americanus* (Walbaum) (Hunt von Herbing, 2001). The existence of such species-specific patterns within the same group indicates that there is a genetic basis for side of eye development, though the developmental

basis for left-right asymmetry has not been addressed in flatfishes (Bolker & Hill, 2000).

Tail growth in California halibut larvae was characterized by a synchronous allometric growth in length and height from hatching to metamorphosis. This increase in tail length at 6.0 mm L_S was concomitant with the differentiation of primordial finfold into unpaired fins and completion of notochord inflexion at 5.7–6.9 mm L_S (23–25 dph). Similarly to *P. olivaceus* (Seikai *et al.*, 1986), the abrupt increase in tail height in California halibut detected from 4.9 to 6.7 mm L_S (17–25 dph) seemed to be correlated with the development of epiaxial and hypaxial musculature, resulting in a considerable change in body shape from an elongated preflexion larva to a more robust flexion specimen. The replacement of the primordial finfold by dorsal and anal fins, the development of the caudal peduncle, and the transition from an anguilliform to a subcarangiform swimming mode as a consequence of notochord flexion are valuable strategies reducing the high costs of fish larval locomotion and increase swimming efficiency (Blaxter, 1988; Osse, 1990). These changes should result in an improvement of larval swimming capabilities (Webb & Weihs, 1986) and feeding success (Blaxter, 1988). Also, transport costs decrease rapidly with growth, being up to five times higher in larvae than in older stages of development (van Snik *et al.*, 1997).

The negatively allometric growth of the trunk during the first stages of development in California halibut larvae was not surprising, as it is common for most fish species to develop the anterior and posterior body regions prior to the abdominal region. This ensures that the essential organs for primary functions (feeding, respiration and locomotion) are developed first (Osse & van den Boogart, 1995). Although the larval digestive system developed and gut coiling proceeded, trunk growth in length decreased from 24–25 dph to the completion of metamorphosis as the abdominal region was compressed during the metamorphic changes. This reduction in trunk growth is different from bilateral fish species, where it increases after the development of the head and tail (Osse *et al.*, 1997; van Snik *et al.*, 1997; Gozlan *et al.*, 1999). In flatfishes this compression of the abdominal region seems to be associated with the acquisition of a definitive asymmetrical body shape.

In conclusion, organogenesis and differentiation in California halibut larvae were more rapid and complex during the preflexion phase of development, as larvae developed most of their sensorial, feeding, respiratory and swimming systems. After notochord flexion most morphological changes were related to the progressive transformation from a bilateral symmetrical larva to a bilateral asymmetrical juvenile. Different changes in body size and proportions were concomitant with changes in the development of anterior and posterior body regions, and in swimming and feeding behaviour. The morphological staging scheme and behaviour described for California halibut from hatching to metamorphosis can be used to facilitate experimental research for developing the intensive culture of this species.

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