

Ontogenetic and Ecological Control of Metamorphosis Onset in a Carapid Fish, *Carapus homei*: Experimental Evidence From Vertebra and Otolith Comparisons

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ABSTRACT In *Carapus homei*, reef colonisation is associated with a penetration inside a sea cucumber followed by heavy transformations during which the length of the fish is reduced by 60%. By comparing vertebral axis to otolith ontogenetic changes, this study aimed (i) to specify the events linked to metamorphosis, and (ii) to establish to what extent these fish have the ability to delay it. Different larvae of *C. homei* were caught when settling on the reef and kept in different experimental conditions for at least 7 days and up to 21 days: darkness or natural light conditions, presence of sea cucumber or not, and food deprivation or not. Whatever the nutritional condition, a period of darkness seems sufficient to initiate metamorphosis. Twenty-one days in natural light conditions delayed metamorphosis, whereas the whole metamorphosis process is the fastest (15 days) for larvae living in sea cucumbers. Whether the metamorphosis was initiated or not, otoliths were modified with the formation of a transition zone, whose structure varied depending on the experimental conditions. At day 21, larvae maintained in darkness had an otolith transition zone with more increments (around 80), albeit wider than those (more or less 21) of individuals kept under natural lighting. These differences in otolith growth could indicate an increased incorporation rate of released metabolites by metamorphosing larvae. However, the presence of a transition zone in delayed-metamorphosis larvae suggests that these otolith changes record the endogenously-induced onset of metamorphosis, whereas body transformations seem to be modulated by the environmental conditions of settlement. *J. Exp. Zool.* 301A:617–628, 2004. © 2004 Wiley-Liss, Inc.

INTRODUCTION

Many fish undergo a metamorphosis which ends their larval period and begins their juvenile period (Youson, '88). The length of this transition phase could be linked to the magnitude of the metamorphosis (McCormick and Makey, '97). The metamorphosis can be spectacular in certain groups like Lampreys, Anguilliform leptocephalous larvae or Pleuronectiforme larvae (Pfeiler, '86, '99; Youson, '88). Various studies have shown that metamorphosis is initiated under the action of both environmental factors (such as habitat shift) and endogenous factors on which relies the aptitude of larvae to metamorphose (Bertram et al., '93; Amara and Lagardère, '95). The most conspicuous criterion is size which accounts, as a

whole, for anatomical (organogenesis *sensu stricto*), metabolic, and endocrine processes. After manipulating the thyroid status of larvae, it was also shown that regulation via the pituitary-thyroid axis plays the primary role in the metamorphosis process (Manzon et al., '98).

As emphasised by Markle et al. ('92), the concept of competency refers to the ability to settle, which can, or cannot, occur during fish metamorphosis (Youson, '88). In most coral reef

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fish, larvae are transformed into juveniles when they settle in a lagoon. These fish possess a complex life cycle involving a pelagic dispersion period of larvae and a benthic life period acquired after the colonisation of the coral reef crest and settlement in the lagoon (McCormick and Makey, '97; Leis and McCormick, 2002). For these species, this shift of habitat is a crucial phase leading to important behavioural changes, especially in feeding habits, niche choices, and predator escape.

Species of the Carapini tribe (Carapidae, Ophidiiformes) have the ability to penetrate and live inside different invertebrate hosts such as sea cucumbers, sea stars, or bivalves (Markle and Olney, '90; Parmentier et al., 2000). Their life cycle may be divided into four stages: (1) the vexillifer larva stage corresponding to the dispersal pelagic period and characterised by a complex specialisation of the dorsal fin called *vexillum* (Olney and Markle, '79; Govoni et al., '84); (2) the *tenuis* stage, marked by the loss of the *vexillum* and by a considerable lengthening of the body, ends when the fish settles; (3) the juvenile stage, reached after the *tenuis* has undergone a significant reduction in length (Smith et al., '81; Markle and Olney, '90), which gives it an adult-like morphology; (4) the adult stage. According to Markle and Olney ('90) and Parmentier et al. (2002b) *tenuis* larvae of *Carapus homei*, *Carapus boraborensis*, and *Encheliophis gracilis* undergo a reduction in body length of about 60% while shifting habitat from pelagic life to the lagoon settlement, where fish find their holothurian hosts for the first time. In vertebrates, the Carapini metamorphosis seems to be unique for at least two reasons. It occurs once the fish is ready to conquer a new environment. It implies a progressively increased decalcification of all vertebral centra along a postero-anterior gradient, the complete disappearance of the posterior vertebrae and associated structures, and an important shortening of vertebral centra, from the anterior to the last remaining ones (Parmentier et al., 2004).

The Carapini otoliths (Parmentier et al., 2002a) have a pattern common to teleost fish (see Panfili et al., 2002 for terminology used in otolith studies): they grow by the alternate deposition of matrix-dominant (D-unit) and mineral-dominant (L-unit) layers according to a specific process leading to a species-specific form (Nolf, '85; Parmentier et al., 2001). The otoliths of the larvae caught at their entrance on the reef show discontinuities ("landmarks") linked to the metamorphosis and/or to the settlement (Wellington and Victor, '92; Wilson and

McCormick, '97, '99). Formation of special structures ("checks") and variations in width of increment units also provide various information on the way of life of the fish (Campana and Neilson, '85; Morales-Nin, 2000). Based on otolith analyses, a delayed metamorphosis of coral reef fish was suggested, which could increase the probability of meeting optimal conditions for settlement (Victor, '86; McCormick, '94, '99). Parmentier et al. (2002a) have described similar patterns in the sagittal otoliths of *C. homei*, *C. boraborensis*, and *E. gracilis*. These sagittae encompass three main ontogenetic zones. The first zone develops during the pelagic life, which ends when *tenuis* larvae enter the lagoon (see also Parmentier et al., 2002b). The second zone, characterised by the presence of low-contrast and wider increments, corresponds to the metamorphosis of Carapidae. It ends with a *post-metamorphic mark* beyond which the development of a third zone corresponds to the juvenile and adult periods.

Carapus homei (Richardson, 1844) possesses all the above mentioned characteristics, which enable us to experiment on the effects of light, host, and feeding. The aims of this study were to more accurately describe the metamorphosis of carapid species and to identify, if possible, environmental cues liable to act on the process. A particular focus was given to changes observed in vertebral centra and otoliths. *Tenuis* larvae were caught during the settlement phase and set in different experimental conditions.

The specific objectives were to relate metamorphic events with the structural changes of vertebral axis and otoliths in order i) to investigate whether catabolic processes, due to regression of vertebrae during *tenuis* transformation, could interfere with otolith formation and (ii) to discuss to what extent larvae could delay metamorphosis.

MATERIALS AND METHODS

Larvae of *Carapus homei* were collected when they arrived on the reef crest, Day Post Capture (DPC) 0 = settlement (Table 1), with a net similar to the one used by Dufour et al. ('96). They were captured at the *tenuis* stage from two different coral reef sites. The first was in front of the Opunohu bay, Moorea, French Polynesia. Six *tenuis* larvae were caught during June, 2001, and 20 were caught during December, 2001. The second site was situated in a "hoa" (small pass between the ocean and a lagoon) on the Rangiroa atoll (French Polynesia). A set of 15 *C. homei* were

TABLE 1 Morphometric and meristic data of sea-caught (# 1–5) and reared under experimental conditions (# 6–27) *tenuis* larvae. All specimens were ranked according to their developmental status (DVS), from pre-metamorphic stage A, to late metamorphosing stage F. EC: experimental conditions (see text for details); HL: head length; BLC: body length at capture; BL: body length at the end of the experiment; SAL: sagitta length; VERT: number of vertebral bodies; NA: number of neural arch; DPC: number of days post capture=calendar days since settlement

	EC	HL	BLC	BL	SAL	VERT	NA	DPC	DVS
1	AN	3.88	21.0	21.0	0.65	181	6	0	A
2	AN	4.08	24.2	24.2	0.63	186	6	0	A
3	AN	4.02	19.7	19.7	0.67	180	6	0	A
4	AN	4.02	19.4	19.4	0.71	182	6	0	A
5	AN	4.12	19.6	19.6	0.66	180	6	0	A
6	SF	4.15	18.7	18.6	0.66	180	7	7	B
7	SF	3.8	18.6	17.5	0.67	174	7	7	B
8	DL	3.92	19.7	18.3	0.72	171	7	7	B
9	DL	3.8	19.4	17.6	0.67	171	7	7	B
10	DLS	4.00	19.7	18.7	0.68	170	7	7	B
11	DLO	3.88	19.7	17.5	0.72	172	6	14	B
12	DLS	4.12	20.0	19.0	0.68	161	7	9	B
13	DLO	4.00	19.8	19.7	0.73	170	6	14	C
14	DK	4.30	19.3	16.0	0.75	162	10	7	C
15	DLS	4.30	19.6	15.0	0.73	156	11	21	C
16	DKO	3.96	18.5	14.5	0.72	142	15	9	D
17	DLO	4.12	19.7	15.3	0.85	165	19	21	C
18	HT	4.90	19.6	14.3	0.92	164	30	7	D
19	DK	4.20	17.3	13.1	0.85	138	11	21	D
20	DKO	4.80	20.0	11.0	1.01	?	37	11	E
21	SF	4.95	18.6	13.5	1.00	133	26	14	E
22	HT	5.31	22.0	10.0	1.08	?	20	11	E
23	SF	5.31	18.5	10.5	1.16	?	36	14	E
24	HT	4.88	19.4	7.5	1.27	119	91	14	E
25	SF	5.40	18.5	8.8	1.27	115	105	14	E
26	HT	6.40	19.6	8.2	1.93	119	119	21	F
27	HT	6.68	19.7	8.2	2.38	121	121	21	F

fished during the month of July, 2002. In total, 27 of these larvae were used in the framework of this study. All larvae were measured for total body length (BLC) immediately after sampling (Table 1). A sub-sample of five individuals from both sites was selected to be deeply anaesthetised (AN) with MS222 before storage in an ethanol solution (75%), awaiting further analyses.

The other 22 fish were kept under various experimental conditions (EC), in tanks filled with running sea-water. For those samples maintained under natural lighting conditions, photoperiod was 13L: 11D. The seven following experimental designs were set, depending on the abiotic or biotic stimuli to be tested.

1) Daylight (DL): tank filled with sea-water, under natural lighting conditions for experiment durations of 7 and 21 days.

2) Daylight + odor (DLO): sea-water filling a tank divided in two parts by an opaque surface with holes which separated the fish from a sea-

cucumber (*Bohadschia argus*); natural lighting conditions for experiment durations of 7, 14, and 21 days.

3) Daylight + sight (DLS): same experimental design except for the use of a transparent dividing wall to separate the fish from the same sea-cucumber species (experiment durations of 7, 14, and 21 days).

4) Dark (DK): tank filled with sea-water, shaded from natural daylight for experiment durations of 7 and 21 days.

5) Dark + odor (DKO): tank filled with sea-water, containing one holothurian (*Bohadschia argus*) and a fish kept inside an opaque PVC tube with perforated walls at each end; experiment durations of 9 and 11 days.

6) Shelter + food (SF): tank filled with sea-water in which small grey PVC tubes enabled fish to freely find shelters; fish were fed with *Artemia nauplii* and various leptocephalous larvae; experiment durations of 7 and 14 days.

7) Holothurid (HT): tank filled with sea-water, presence of holothurian hosts (*Bohadschia argus*), which allowed fish to penetrate into them; experiment durations of 7, 11, 14, and 21 days.

At the end of each experimental condition, all the fish were deeply anaesthetised with MS222. All the specimens were stained with alizarin Red S according to Taylor and Van Dyke's method ('85). The coloured fish were examined with a Wild M10 binocular microscope coupled to a camera lucida. The following data were collected for each specimen: body and head lengths (BL and HL, measured to the nearest 0.1 mm, from the mesethmoid to the end of the tail, and from the mesethmoid to the end of the basioccipital), otolith (sagitta) length taken along the antero-posterior axis (SAL measured to the nearest 0.01 mm), numbers of vertebrae (VERT) and of neural arches (NA) stained by alizarin. In addition, more detailed observations were collected for each individual: the vertebra from which were noticed the early beginnings of the decalcification process and the number of noncalcified vertebrae.

Optical frontal sections of sagittae were prepared by mounting each otolith on a glass slide in thermoplastic cement (Parmentier et al., 2002a). Sections were obtained by grinding each slide with wet sand papers of decreasing size (600, 800, and 1200 grit) and polishing them with wet 0,3 µm grit

paper. Preparations were observed and photographed using a polarised light microscope (Olympus BX50), fitted with an Olympus OM-4 Ti photo camera. All increments deposited in zone 2 that developed after capture were counted, irrespective of the numbers of days elapsed since day 0 (= day of capture, DPC 0).

RESULTS

From skeletal and otolith characteristics of metamorphosing *tenuis* larvae, six ontogenetic stages (A to F, Fig. 1 and Table 1) or larval developmental status (DVS, Table 1) were established, according to variables as body length, head length, sagittal otolith length, number of neural arches, and vertebrae. The responses to staining of vertebrae were also taken into account. The 6 stages are presented below.

Metamorphic developmental stages

Spinal column

Stage A. *Tenuis* of *C. homei* show a greyish deciduous whip owing to the numerous covering melanophores at their caudal tip (Fig. 2A). This 30 mm long whip exhibits neither cartilage, osseous matrix, nor fin-ray bearers. It looks like a fibrous appendix added to the last vertebra. It is easily

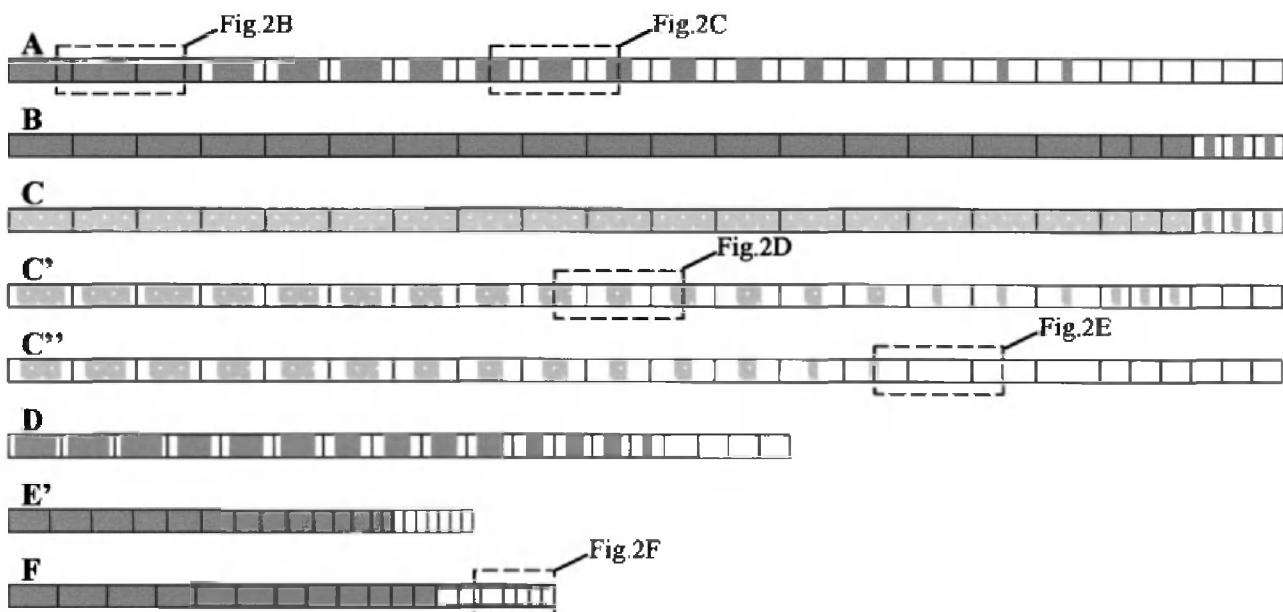


Fig. 1. Schematic representation of the vertebral bodies of the column in the *tenuis* larva during metamorphosis. The annotations A to F correspond to the development stages described in the text and table 1. The squares refer to the illustrations in Figure 2. The shadowed areas represent the calcified part of the vertebra, the shadowed dotted areas are zones in decalcification and the white areas are nonossified vertebral bodies.

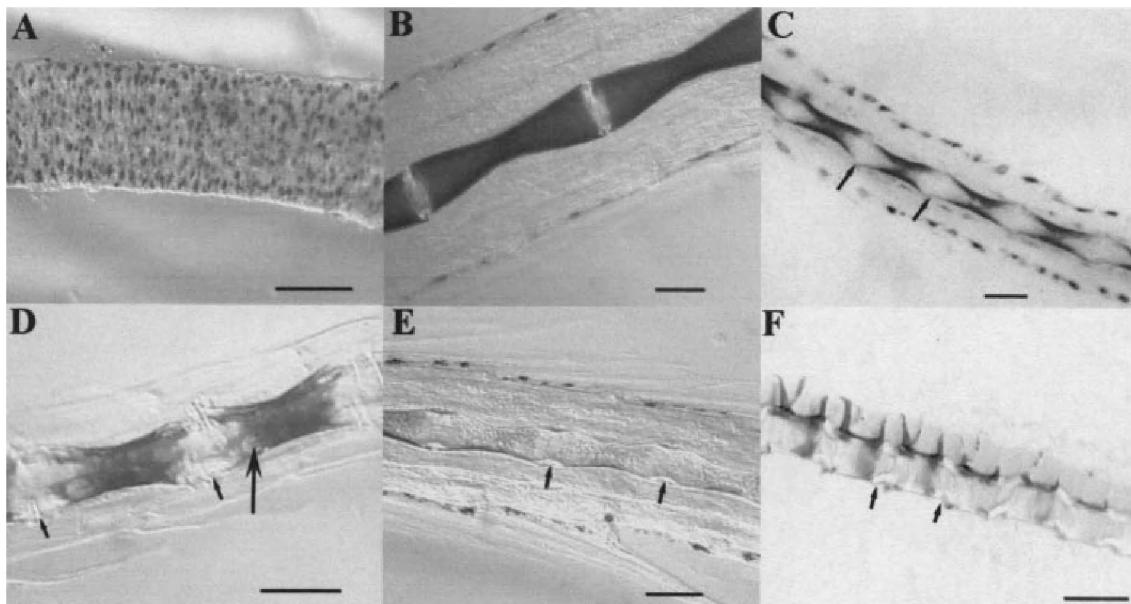


Fig. 2. **A.** Lateral view of the caudal whip in a tenuis larva at the time of settlement. **B to F:** lateral views of the vertebrae at different development stages (see Fig. 1). Scale bar = 0,2 mm. Small black arrow: length of vertebral bodies. Large black arrow: decalcified zones.

removed off the body and seems to be the first element responsible for the reduction of fish length. Tenuis caught at their arrival on the reef have a suite of 174 to 186 vertebrae (Table 1). The lengths of the 130 first vertebrae range between 0.8 mm and 1.2 mm. Starting at the 130th vertebra, vertebra length diminishes progressively to 0.4 mm at the caudal tip. Depending of the specimens, the 100 to 140 first vertebrae have the shape of a completely alizarin-stained diabolo (Fig. 2B). The alizarin staining is restricted to a single central band on the remaining vertebrae (Fig. 2C). Towards the caudal tip, this central band becomes progressively narrower until its complete absence on the last vertebrae (Fig. 1A). It is, however, still possible to distinguish the osseous matrix in these vertebrae. At this stage of development, only the neural arches of the six first vertebrae are alizarin stained; all the remaining vertebrae possess cartilaginous neural arches with a thin rod shape.

Stage B. In this group of larvae, the alizarin-stained central band seems larger for all the concerned vertebrae (Fig. 1B). It seems that there is a completion of the calcification process initiated before the colonisation of the reef. Nevertheless, it is possible to observe a few incompletely stained vertebrae at the caudal tip.

Stage C. The larvae of this group show vertebrae pricked with whitish spots (Fig. 1C, 2D), which seem to indicate a poorer fixation of the alizarin. This corresponds to a decalcification process. The

latter begins simultaneously at each tip of the vertebral body and seems to converge toward the centre. Depending on the specimen, the number of vertebrae showing only a thin alizarin-stained central band varies from 16 to 65 (Fig. 1C', 1C''). Behind these vertebrae, there remain 65 to 67 vertebrae that seem completely uncalcified (Fig. 2E).

Stage D. In this group, fish possess totally stained vertebral bodies that are distinguishable from those of group B because of their shorter vertebral lengths and less numerous vertebrae (Fig. 1D). These fish also possess a greater number of alizarin-stained neural arches (Table 1).

Stage E. At this stage, the vertebral column is made of a small number of shorter vertebrae. It all seems as though the vertebra had undergone a process, analogous to the one observed in genetic, that could be called an "excision-splicing": the nonstained vertebral body parts are eliminated and the stained parts are brought closer together (Fig. 1E). The smallest fish measures 7.5 cm and has 119 vertebrae, but not all of the neural arches are alizarin-stained.

Stage F. The fish of this group are different from the former by their caudal tip vertebrae where the calcification of the neural arches appears before that of the vertebral bodies. The latter show either an osseous matrix, or a start of calcification. Unlike the vertebrae of the former groups, calcification begins by the dorsal and ventral

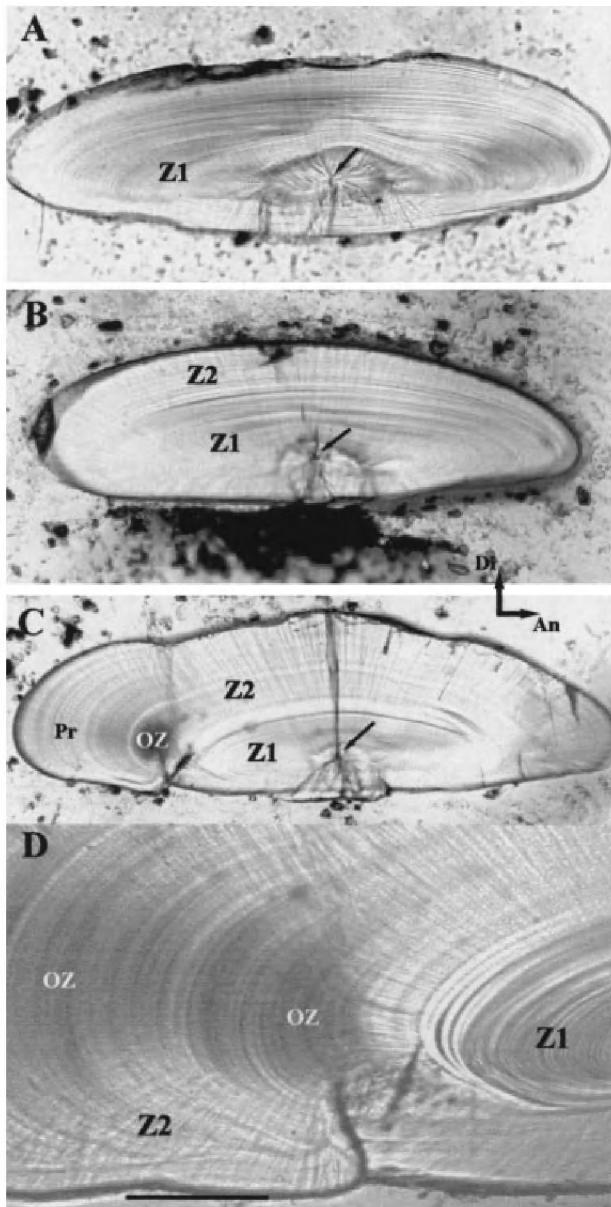


Fig. 3. Frontal sections of right sagittae. A: at the time of settlement (length: 0,65 mm); B: after 7 days under DK conditions, showing the onset of zone 2 - (length: 0,75 mm); C: situation after 21 days for a *tenuis* placed in a holothurian (length: 1,93 mm); the change in shape is due to the post-rostrum formation (Pr) and an opaque zone corresponding to the important concentration of organic material; D: situation after 21 days for a *tenuis* placed in a holothurian; zoom on the opaque zone, Scale bar: 0.1 mm. An: anterior part, di: distal part, OZ: opaque zone, Pr: postrostrum, Z1: zone 1; Z2: zone 2; black arrow: nucleus.

margins of the vertebral bodies and not by a central band (Fig. 2F). In comparison with the group E, the size of the vertebrae increases once again (Fig. 1F). Compared to group A larvae, F fish (#26 and 27) lose approximately 60% of their size

and 38% of their vertebrae. They also have a cranium 1.7 longer than the cranium of A larvae.

Sagittal otolith comparisons

Stage A larvae (Table 1) caught when settling in the reef have sagittae with typical zone 1 structures corresponding to the pelagic period of life (Fig. 3A), i.e. made of thin increments deposited around the core (Parmentier et al., 2001, 2002a). The otolith possesses an elongated elliptic shape following the anterior-posterior axis, and is flattened at the proximal face side.

All the larvae used in the different experimental conditions have otoliths that show zone 1 (Z1) and a second zone (Z2, Fig. 3B and 3C) which corresponds to the metamorphosis stage. Generally speaking, the zone 2 was more opaque than zone 1 due to the wider D-units of increments constituting zone 2 (Fig. 3D). However, the latter showed various patterns, depending on the conditions experienced by larvae.

Sagittae of larvae settled in holothurians had the widest and the most numerous increments (Fig. 3C). On that account, the whole sagitta thickens and lengthens towards the posterior with the formation of the typical post-rostrum (Pr, Fig. 3C) of the Carapini (Parmentier et al. 2001). The innermost posterior part of zone 2 was characterised by an opaque spot (OZ, Fig. 3C) hiding the alternation of D- and L-units. However, this spot did not conceal any accessory growth centre, as shown at higher magnification (Fig. 3D), and the darkness of this spot was probably due to increased organic material deposited in the wider D-units. The number of increments in the zone 2 of the sagittae was not determined with precision but it appears to be largely superior to the number of days the fish spent inside the sea cucumber: above 80 for 21 days (#26 and #27), 50 for 14 days (#24), and above 25 for 7 days (# 18). Conversely, the larvae reared in daylight (DL, DLO, DLS) have deposited narrower increments in zone 2 (DLO, Fig. 4A), in equal or almost equal number to that of the experiment duration. Between these two extremes, fish reared in darkness (DK, Fig. 4B) or those that were fed (SF) have a greater number of increments than the number of experimental days in their zone 2. However, fish in SF have more numerous and larger increments than those in DK. The post-metamorphic mark described in Parmentier et al. (2002) and separating zone 2 from zone 3 was never observed in any sagittae of this study.

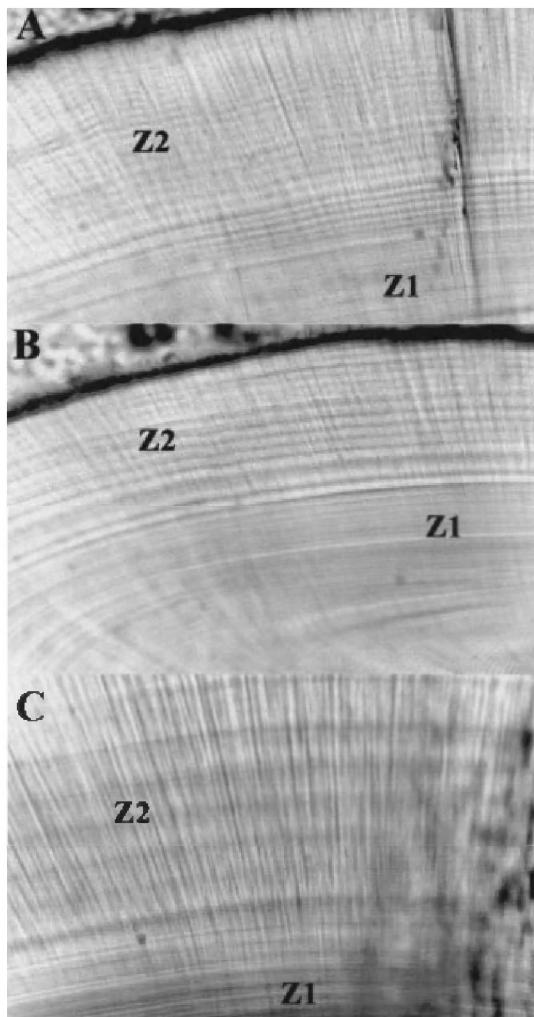


Fig. 4. View of the frontal sections of the right sagittae in larvae placed in different experimental situations. **A:** fish kept 21 days in DLO; **B:** fish kept 7 days in DK; **C:** fish kept 21 days in a holothurian (HL). These images taken at the same magnification clearly show the existing differences in the width of the increments. Z1: zone 1; Z2: zone 2. opaque band corresponds to D-unit and clear band to L-unit.

Comparison between experimental situations

Light vs. darkness effects

At the same number of days post capture (DPC), the less developed fish were the fish reared in daylight, next to a sea cucumber or not. In other words, if two fish have the same development status, it appears that the one reared in daylight is older. At DPC 7, fish #14 (status C) possessed more neural arches, fewer vertebrae, and had a shorter body with a more developed skull and otoliths than fish which spent the same time in daylight (#6 to #10, status B). On the other hand,

fish #16 (status D) had the same developmental status as fish #15 and #17 (status C) but they are respectively 9 and 21 DPC. These data suggest the existence of a direct influence of light (or its absence) on the initiation of the carapid development.

Fish-host interaction

Under darkness conditions, fish allowed to perceive the odor of an holothurian exhibited a faster body transformation than those only kept in darkness. Fish #20 (status E) appears to be more developed than #19 (status D), whereas the first is 11 DPC and the second is 21 DPC. On the other hand, the odor of the sea cucumber did not seem to have any influence when the fish were reared in daylight. Fish reared inside the sea cucumber or fed in the aquariums provided with shelters (SF) were the most developed. However, there is a difference between the two experimental conditions. The tenuis (#18) living inside the sea cucumber is 7 DPC and is at the development state D whereas the tenuis (#6 and #7) reared in SF show a delay (B) after the same lapse of time.

For *C. homei*, the sea cucumber could principally be considered as acting as a black box. However, the odor of the sea cucumber could also have a role in the development if it is associated with darkness: the fish reared in DKO are more developed than in DK.

DISCUSSION

Manipulations of *C. homei* larvae at the tenuis stage, under various experimental situations, give us evidence that both fish ontogeny and otolith structural changes could be impaired by metamorphosis conditions. These elements allow us to underline the particularities of a metamorphosis that seems, to the best of our knowledge, unique in fish. The effects on calcified tissues varied, depending on whether skeleton or otoliths are concerned, and could entail a delayed larval transformation but not an interruption of the otolith growth. The stages and conditions of this metamorphosis are discussed in three points that 1a) clarify the scheme of metamorphosis at the vertebral column level and 1b) at the otolith level, and 2) consider factors that initiate the metamorphosis.

Reappraisal of the metamorphosis scheme

According to Parmentier et al. (2004), carapid metamorphosis could be schematically explained

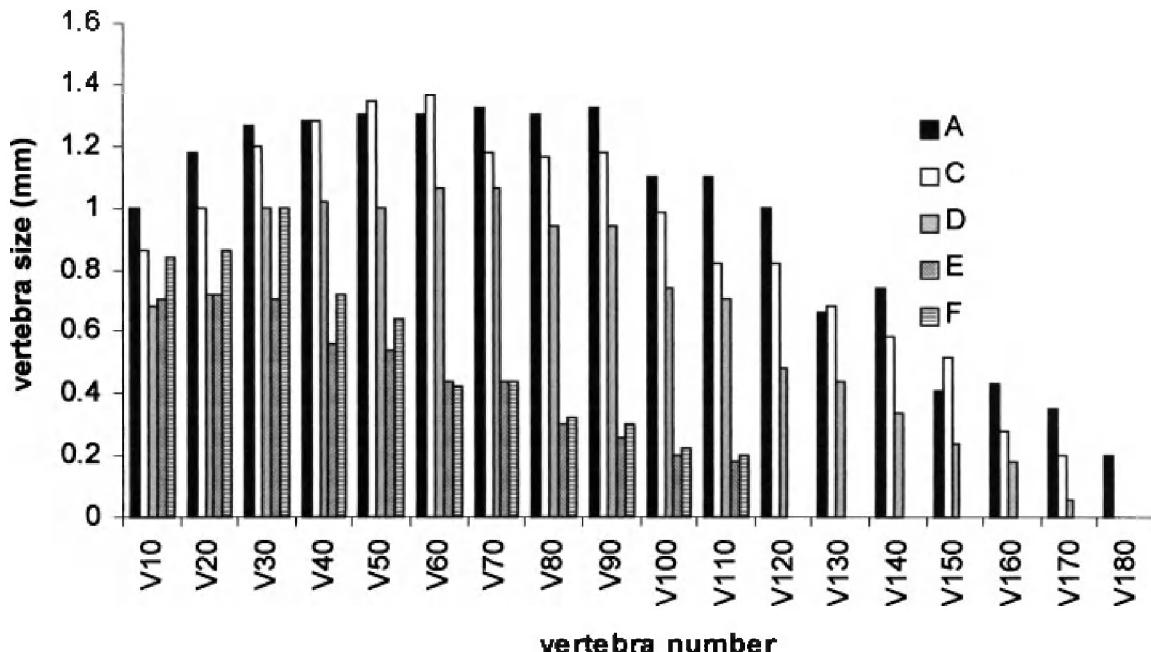


Fig. 5. Changes in length of vertebral bodies during metamorphosis. **A** to **F** correspond to the different development status (see text). v# = serial number of the vertebra.

in terms of gradients. First, the reduction in both the number and size of vertebrae follows an increasing *decalcification*, *histolysis*, and *shortening* gradient. This entails a complete histolysis of the caudal tip vertebrae, whereas the anterior vertebrae do not seem to be under a marked influence from this first gradient. On the other hand, the morphological modification of the axial skeleton from the juvenile to the adult suggests the existence of another *constructive* gradient. The latter decreases gradually towards the caudal tip and builds the vertebral axis in a series of vertebrae that become progressively shorter and less complete.

From skeletal and developmental characteristics, this study gives us deeper insights on the succession of events involved in the metamorphosis. Comparisons between all the ontogenetic stages would suggest the existence of two development axes:

axis 1)

A → C → D/E → F

axis 2)

B

The first axis describes a developmental path that closely resembles natural conditions. According to the axis 1, the metamorphosis is marked by a progressive reduction of the vertebral body size and a reduction of the total number of vertebrae (Fig. 5). The larvae arriving on the reef (A) lose their distal tip. They have a series of vertebrae

whose size and calcification level diminish towards the posterior end. During stage C, this decalcification process expands and leads to the decalcification of the last 80–90 vertebrae following a posterior-anterior gradient. This decalcification is partial at first, but becomes complete at the distal tip. In the following step (D/E), the “excision-splicing” process leads to an important size reduction by both the shortening of the vertebrae size and loss of the caudal tip vertebrae. The development goes on with a new vertebral growth and the completion of the ossification of the neural arches (F), a process already initiated in C. This axis 1 (A to F) is also marked by the neurocranium and sagitta growth which appears to accelerate at the D/E stage. The second development axis (with an additional stage B) seems to be induced by particular experimental conditions. Fish that are not reared in natural conditions (i.e. inside sea cucumber) show a delay in the initiation of metamorphosis, which could explain the reinforcement of calcification of the vertebral bodies. The axis 2 is however completed, as the first one. This could indicate that one or several factors which initiate metamorphosis were affected but not the process itself.

Otolith vs. development chronologies

Otolith structure and composition are biological archives of both ontogenesis and fish living

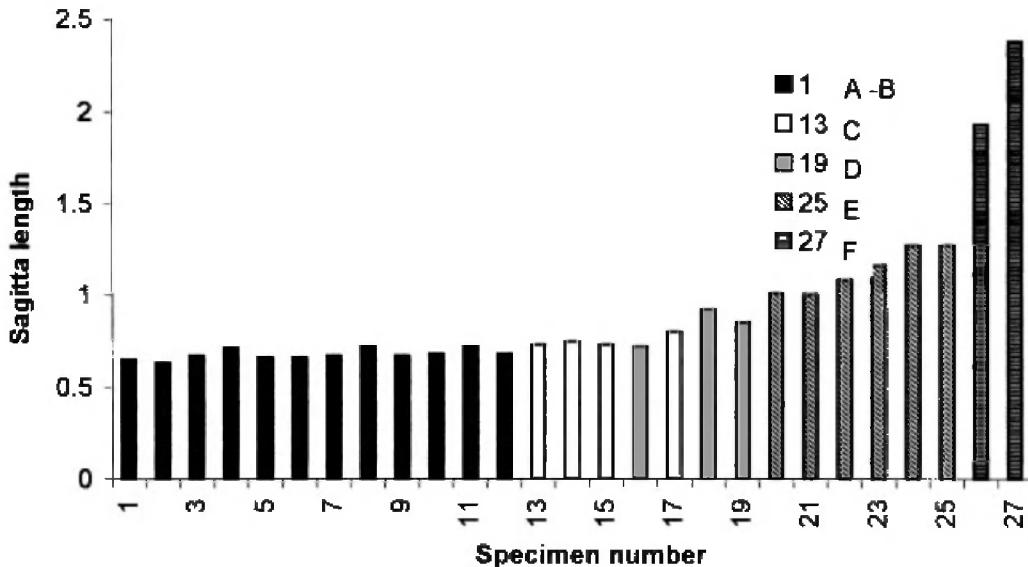


Fig. 6. Increase in length of the sagitta during metamorphosis. A to F correspond to the different development status (see text). Numbers refer to the fish record given in Table 1.

conditions (Gauldie and Nelson, '90; Morales-Nin, 2000). A structural otolith discontinuity (*settlement mark*) was identified in various coral reef fish when they shift from pelagic life to benthic life (Victor, '86; Wellington and Victor, '92; Wilson and McCormick, '99). It has been shown in teleosts characterised by profound metamorphic changes that the shift of habitat coincides with the initiation of metamorphosis. For example, the sagittae of leptocephali (e.g., conger *Conger myriaster*, eels *Anguilla anguilla* and *A. rostrata*) form a transition zone recording that the leptocephalous larvae leave the ocean and approach the continental shelf or that they initiate metamorphosis or both (Lee and Byun, '96; Cieri and McCleave, 2000). A complex transition zone is also present in the otolith of other fish that have a progressive transformation, e.g., gadids as cod *Gadus morhua* (Lagardère et al., '95) and the sand-eel *Ammodytes marinus* (Wright, '93) or a deep metamorphosis like the Pleuronectiformes (Sogard, '91; Modin et al., '96; Lagardère and Troadec, '97; Amara et al., '98).

The larvae of *Acanthurus triostegus* (Acanthuridae) caught at the time of settlement on the reef and artificially replaced in the pelagic milieu were able to delay metamorphosis (McCormick, '99). Their otoliths possessed however the same structural modifications than those of fish reared in benthic cages where metamorphosis was correctly completed. In *C. homei*, with the exception of the larvae caught at the settlement, all the otoliths developed the zone 2, whatever the experimental

conditions and the time elapsed from capture. This study experimentally confirms that the formation of the transition zone is not induced by environmental variations linked to the settlement but depends on an internal signal since the zone 2 is present in tenuis reared in natural lighting and which showed a delayed metamorphosis.

However, the structure of the zone 2 varied depending on the metamorphosis progress. Under opposite rearing conditions (holothurian host vs. daylight for 21 days), newly metamorphosed juveniles and stage C larvae were obtained (for example larvae #27 and #17, respectively). Although juvenile #27 underwent an almost two-fold body size reduction, its otoliths were 2.27 times longer than those of #17 (Fig. 6). In other words, the more the body had shortened, the bigger were the otoliths. Moreover, the larvae for which the decalcification process was initiated, also possessed a transition zone with a number of increments superior to the number of experimental days. This suggests that fish could benefit from the material released by body reduction in order to use it in the development of the bones and the otoliths. The otoliths and the bones have different calcification modes but their growth depends on the calcium level of fish plasma (Pannella, '80; Mugiyama, '84; Toshe and Mugiyama, 2002).

Moreover, a correlation between the otolith formation and the body (or the skull) development is usually observed (Campana and Jones, '92;

Wang and Tzeng, 2000; Parmentier et al., 2001). In fish otoliths, calcium incorporation seems to proceed through a trans-epithelial pathway via specialized cells of the otolithic membrane supplied by blood capillaries (Payan et al., '99, 2002). Accordingly, a strong correlation exists between the calcium level of fish plasma and the calcium deposition rate on the otolith (Mugiyama et al., '81; Campana, '83a). Calcium does not seem to be a limiting factor of otolith formation in marine teleosts. It results principally from absorption via the gills (Mugiyama et al., '81; Campana, '83a). However, some skeleton elements could also constitute a reusable Ca^{2+} stock. For example, resorption of larval teeth and of the gelatinous matrix in leptocephalous larvae release Ca^{2+} that is used in the formation of the skeleton (Pfeiler, '97); the salmon *Salmo salar* undergoes a demineralisation of the vertebral column, the calcium of which is used for the remodelling of cranial bones for sexual maturation (Kacem et al., 2000). In *C. homei*, the demineralisation of the vertebral bodies could enhance calcium levels required for the formation of calcified structures.

This reuse of metabolites also seems to result in a broadening of the matrix-dominant D-units. This results in a more opaque increment deposition, which could correspond to a denser mesh of organic fibres (Pannella, '80). Studies have shown a positive correlation between the food-supply level and otolith growth (Campana, '83b; Molony, '96; Morales-Nin, 2000). In different species of coral reef fish that do not feed during the metamorphosis (Leis and McCormick, 2002), there is a diminution in the size of the increments (Wilson and McCormick, '99). This is not the case in *C. homei* where a transition zone develops both in fed and starved fish. This reinforces the hypothesis of a re-investment of organic material released during vertebral axis reduction.

Delay of metamorphosis

Before this study, the experimental demonstration of a delayed metamorphosis in a coral reef fish was only realised for the Manini *Acanthurus triostegus* (McCormick, '99), study which does not deal with factors that could have an influence on metamorphosis. In fish, the latter could be controlled by both environmental and hormonal factors (Youson, '88). The comparison of the somatic metamorphosis with the structural variations of the otolith is in good agreement with the

study of McCormick ('97) on the Manini. Fish caught when they settled on the reef did not have a settlement mark. The latter is however recorded afterwards, whatever the tested experimental condition and even if the fish had not initiate its metamorphosis. This could indicate a decoupling between otolith and somatic developments. During metamorphosis, changes in otolith structure rely above all on an internal cue, whereas body transformation also depends on environmental conditions. In the case of *C. homei*, the appropriate environmental conditions seem to be principally linked to light.

The type of internal message appears more difficult to determine. The metamorphosis initiation has been shown to be size-dependant in various teleosts (Youson, '88), as if fish has to raise its '*capacity for mature function*' (Bertram et al., '93), namely functional organs and notably the neuro-endocrine system (Chambers and Leggett, '92). For example, settled coral reef fish larvae do not have the same age but the same size (Victor, '86). In various larvae of flatfish and leptocephali, metamorphosis is mediated by thyroid hormones (e.g., Youson, '88; Pfeiler, '99). In flatfish larvae, the production of thyroid hormones was shown to synchronize metamorphosis (Gavlik et al., 2002). Darkness induces the melatonin synthesis at the pineal organ level (Ekström and Meissl, '98; Boeuf and Le Bail, '99). In the lamprey, a pinealectomy prevents metamorphosis (Youson, '88), whereas it only affects growth in the Atlantic salmon, *Salmo salar* (Mayer, 2000). This suggests that melatonin could be excessively secreted in prolonged darkness. This could be the origin of a cascade of events allowing the initiation of the metamorphosis. However, these hormonal pathways remain to be studied.

According to McCormick and Makey ('97), the magnitude and duration of the ecological shift occurring during settlement transition could be predicted from information on the extent of metamorphosis: species with a substantial metamorphosis to the juvenile stage have a longer transition period than species that are well developed at settlement. In opposition to the fish studied by McCormick and Makey ('97), *C. homei* larvae do not go through several habitat shifts. This difference is linked to two aspects of the way of life of *C. homei* larvae: they take shelter directly from eventual predators (except cannibalism, Parmentier et al., 2000) and there do not need to actively seek for food.

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