Short note

A photographic guide to the embryonic development of the garfish *Belone belone*

Ein fotografischer Leitfaden für die Embryogenese des Hornhechts *Belone belone*

Vivian Fischbach[1,2,3] & Timo Moritz[1,2,4]

[1]Aquatic Ecology, Institute for Biological Sciences, University of Rostock, Albert-Einstein-Straße 3, 18059 Rostock, Germany, vivian.fischbach@uni-rostock.de (corresp. author)
[2]Ocean Museum Germany, Katharinenberg 14-20, 18437 Stralsund, Germany
[3]Thünen Institute of Baltic Sea Fisheries, Am Hafen Süd 2, 18069 Rostock, Germany
[4]Leibniz-Institute for the Analysis of Biodiversity Change (LIB), Bundesstraße 52, 20146 Hamburg, Germany

Abstract: The garfish *Belone belone* is a marine epipelagic species with a large distribution ranging from the Northern Atlantic to the Mediterranean Sea. It belongs to the order Beloniformes. Beloniform fishes inhabit coastal and marine habitats around the world and thus play crucial roles in several ecosystems. Beloniforms exhibit a complex early life history of which not all developmental phases are fully known yet. This study describes the embryogenesis of the garfish, establishes developmental stages, and provides a photographic guide to identify the different developmental stages.

Keywords: Beloniformes, early life history, embryogenesis, egg development

Zusammenfassung: Der Hornhecht *Belone belone* ist eine epipelagische, marine Fischart mit einem großen Verbreitungsgebiet, das vom Nordatlantik bis zum Mittelmeer reicht. Er gehört zur Ordnung der Beloniformes, welche Küsten- und Meereslebensräume auf der ganzen Welt besiedeln und daher wichtige Rolle in verschiedenen Ökosystemen spielen. Beloniforme Fische weisen einen komplexen Lebenszyklus auf, von denen noch nicht alle Entwicklungsphasen vollständig bekannt sind. Diese Studie beschreibt die Embryogenese des Hornhechts, legt Entwicklungsstadien fest und bietet einen fotografischen Leitfaden für diese.

Schlüsselwörter: Beloniformes, frühe Lebensgeschichte, Embryogenese, Eientwicklung

1. Introduction

The garfish *Belone belone* (Linnaeus, 1761) is a marine epipelagic species of the order Beloniformes comprising two suborders, six families, 37 genera, and at least 230 species which are distributed worldwide (Collette et al. 1984; Collette 2003; Collette & Cole 2010). The garfish exhibits a large distribution ranging from the Mediterranean Sea including Black Sea to the Northeastern Atlantic, including North Sea and Baltic Sea (Collette & Parin 1970). Beloniforms play important roles in various ecosystems as predators (e.g., Collette & Parin 1986; Collette et al. 2018) as well as prey (Jiang et al. 2022). Belonid fishes exhibit a complex life cycle with unique early life stage adaptations (Schlesinger 1909; Nichols & Breder Jr 1928; Collette et al. 1984; Lovejoy & Collette 2001; Collette 2003; Lovejoy et al. 2004). Most fishes

of the genus *Belone,* for example, produce large eggs with attaching filaments which help to link the eggs to surrounding structures such as macroalgae (Collette et al. 1984; Polte & Asmus 2006). Furthermore, during larval development, belonid larvae exhibit characteristic half-beak stages, where at first only the lower jar is developed for specialized feeding (e.g., Collette et al. 1984; Boughton et al. 1991).

In the Western Baltic Sea garfish aggregate for spawning in early spring (Dorman 1988; Dorman 1991). At 18°C garfish embryos hatch after approximately three weeks (von Westernhagen 1974) and the larval and juvenile fish then remain in the shallow coastal areas which function as their nursery habitats (Fischbach et al. 2024). At hatching the larvae already exhibit an almost fully developed postcranial skeleton (Fischbach et al. 2024), suggesting that the majority of developmental processes already occur during the embryogenesis. The embryonic phase of the garfish, i.e. the development until hatching, has been described by several studies to different degrees (Rosenthal & Fonds 1973; Fonds et al. 1974; von Westernhagen 1974; Korzelecka-Orkisz et al. 2005) and the influence of various environmental parameters has been tested (Rosenthal & Fonds 1973; Fonds et al. 1974; von Westernhagen 1974; Alter & Peck 2021). While garfish embryos show distinct temperature thresholds during development between 10°C and approximately 21°C (von Westernhagen 1974), they generally show tolerance to warming scenarios. However, in laboratory studies survival decreased under stable high temperatures and increased pO_2 levels (Alter & Peck 2021). While garfish apparently exhibit a distinct tolerance range for temperature, von Westernhagen (1974) showed, that hatching was almost equally successful at a wide range of salinities (15-33‰). However, the low hatching success below 15‰ indicates, that garfish embryos are potentially at their limit when developing in the shallow coastal areas of the Baltic Sea which mostly exhibit and average salinity of 6-7 PSU (Seifert 1938).

Garfish remain the subject of various studies, and a uniform staging system to allow comparison of embryogenesis or selected developmental processes is still needed. The present study provides a comprehensive and complete photographic guide to the developmental stages of the garfish which serves as a framework for future studies.

2. Materials and methods

Garfish eggs were sampled in the field in the Kubitzer Bodden, the Strelasund and at the periphery of Greifswald Bay in 2022 via beach seine (fig. 1). Additionally, mature adult garfish were caught and strip spawned. Fertilization occurred in the field and first the eggs of the females were spawned into a petri dish and then covered in milt and habitat water and incubated for 15 min. Afterwards the eggs were rinsed with habitat water and transported to the Ocean Museum Germany in wet chambers. At the museum the eggs were incubated in habitat water at ~8.3 PSU at 18°C. The garfish eggs in the different developmental stages were then photographed under a microscope (Leica DMC6200 camera operated with the software Leica Application Suite (version: 3.6.0.20104)) and their development was described and characterized. Since the garfish eggs which were incubated at the museum were largely affected by aquatic pathogens leading to drastically increased mortality, no data on the individual stage duration is available.

3. Results and discussion

The overall embryonic development of the garfish follows the pattern described for fish in general by Miller & Kendall (2009) and can be divided into an early (from fertilization until blastopore closure), intermediate (from blastopore closure until the tailbud is free) and late embryonic phase (from free tailbud until hatching).

When observing the embryonic development of the garfish, distinct stages were characterized when developmental milestones, such as gastrulation, were reached. The observation made in this study are mostly in accordance with observations made by previous researchers investiga-

Fig. 1: Map of Greifswald Bay and its location within the Baltic Sea. Sampling sites for mature adult garfish are marked with black stars.
Abb. 1: Karte der Greifswalder Bucht und ihrer Lage in der Ostsee. Die Fangorte für die erwachsenen, geschlechtsreifen Hornhechte sind mit schwarzen Sternen markiert.

ting the embryogenesis and factors influencing early garfish development (Rosenthal & Fonds 1973; Fonds et al. 1974; von Westernhagen 1974; Korzelecka-Orkisz et al. 2005; Alter & Peck 2021), where stages were e.g., characterized based on age (Korzelecka-Orkisz et al. 2005), however, some distinct differences appeared.

During the early embryonic development, successful fertilization and the beginning of garfish embryonic development was first visible by a patch of small cells at the animal pole (fig. 2a, Stage 1). In other studies, and during lower incubation temperatures also two-cell and 8-cell stages were distinguished (von Westernhagen 1974; Korzelecka-Orkisz et al. 2005), which were not observed in the present study. During this examination, a cluster of cells was already present at first inspection after 24h. Also, all of the eggs sampled in the field displayed a cluster pf cells and no earlier stage was detected, possibly implying that this stage is very short. Next, Korzelecka-Orkisz et al. (2005) described a morula stage characterized by a round cluster of cells at the animal pole. However, we observed that eggs which exhibited this developmental pattern did not continue their development, and we believe that this "stage" rather shows a malformation resulting in the termination of embryogenesis. We found that with increasing cell division at the animal pole, as a next step the blastula cap is formed (fig 2b, Stage 2a) and the cells keep spreading around the yolk (fig. 2c, Stage 2b).

Then during the intermediate embryonic development gastrulation starts and the embryo

Tab. 1: Stages of garfish embryonic development determined in this study in comparison with the description of the stages described by VON WESTERNHAGEN (1974) and KORZELECKA-ORKISZ et al. (2005). The different stage descriptions were put in a timely order and if no concordant descriptions were available in other studies, the cell was left blank.
Tab. 1: Stufen der Hornhecht-Embryogenese aus dieser Studie im Vergleich mit der Beschreibung der Stufen von VON WESTERNHAGEN (1974) und KORZELECKA-ORKISZ et al. (2005). Die unterschiedlichen Stufenbeschreibungen wurden in einen zeitlichen Rahmen eingepasst und wenn keine übereinstimmenden Beschreibungen vorhanden waren, wurde die Zelle leergelassen.

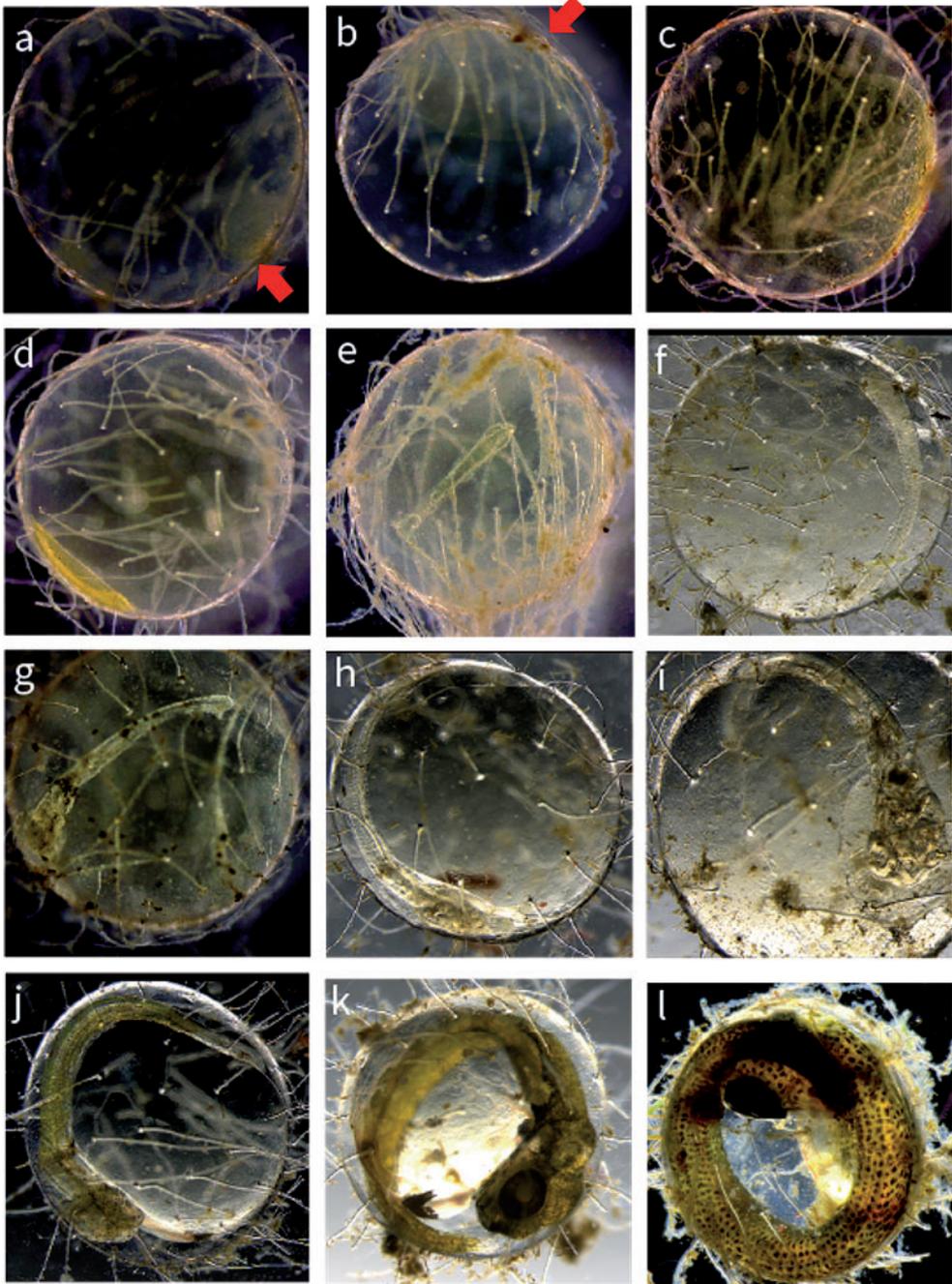| Observations in this study		von Westernhagen (1974)		Korzelecka–Orkisz et al. (2005)	
Stage		Stage			Stage
Early embryonic development - formation of embryo					
				D°: 2.3	Reception mound formation
			2 cell stage	D°:3.3	2 blastomeres
1	The fertilization was successful, and the cell division starts. In this phase only cells are visible			D°:5.6	8 blastomeres
2a	The cells form the blastula cap.	1A	Blastodisc	D°:17	Morula (Fig. 3c,d)
				D°:34	¼ Epiboly
2b	90% epiboly			D°:49	¾ Epiboly
Intermediate development					
3	Gastrulation, embryo is visible on top of the yolk. The embryo already shows myomeres and the lenses and brain develop. Also, the notochord Is visible.	1B	Gastrulation		
		II	Blastopore closed, first pigmentation	D°:71	Blastopore closure; embryo with emerging cephalic part and distinct 4 myomeres in the posterior part
4	The embryo grows and more myomeres develop. The heartbeat is visible, and the blood flow is visible. The embryo reaches around one third of the yolk sac.	III	Heart is beating, appearance of first blood cells;	D°:84	Appearance of egg balls; heartbeat begins
Late development					
5	Tailbud is free, pigmentation starts, and the embryo shows green pigmentation. Later the eyes become pigmented. Also, the skeletal development starts. The caudal fin starts to differentiate first. The embryo now reaches halfway around the yolk sac.	IV	Veins develop on yolk sac, eyes	D°:116	Eyes visible, blood vessel network on the yolk sac, initial movements of the embryo
6	Dorsal and anal fins start to differentiate. The fin buds of the pectoral fins are visible and shortly before hatching the embryo exhibits constant movements of the pectoral fins. The movements of the embryo occur first as small twitching movements and later the whole embryo flips within the egg until it finally hatches. The embryo now reaches at least all around the egg, with the tail and the head (almost) touching.			D°:212	Pectoral fin movements
				D°:324	Opercular movements
		V	50 % of total larvae hatched	D°:340	Hatching

Fig. 2: Embryonic development of the garfish categorized in six developmental stages. **a** Stage 1, **b** Stage 2a, **c** Stage 2b, **d-f** Stage 3, **g-i** Stage 4, **j-k** Stage 5, **l** Stage 6. The red arrows mark the cell cluster in stage 1a and the blastula cap in stage 1b.

Abb. 2: Embryogenese des Hornhechts, kategorisiert in sechs Entwicklungsstufen. **a** Stufe 1, **b** Stufe 2a, **c** Stufe 2b, **d-f** Stufe 3, **g-i** Stufe 4, **j-k** Stufe 5, **l** Stufe 6. Der rote Pfeil markiert den Zellhaufen i Stufe 1a und die Kappe der Blastula in Stufe 1b.

becomes visible on top of the yolk within the former blastula cap and first myotomes are already visible (fig. 2d-f, Stage 3). VON WESTERNHAGEN (1974) chose the end of the gastrulation and thus the closure of the blastopore as a stage characteristic, thus his stage, on a temporal scale, is located between stages 2 and 3 of the present study. In stage 4 (fig. 2g-i), the embryo grows and develops more myomeres. Also, the heartbeat becomes visible approximately when the embryo reaches around one third of the yolk. At approximately this stage, KORZELECKA-ORKISZ et al. (2005) describe the presence of "egg balls", however, no information was given in the exact location of the structure. These "egg balls" could be equal to the large conspicuous vesicles describe by ROSENTHAL & FONDS (1973) which were identified most likely as Kupffers vesicles, a transitory organ which initiates left-right development of the brain, heart and gut in zebrafish (ESSNER et al. 2005). Besides these two accounts, no further mention of similar structures was found in the literature. Also, in the present study no such structures were. It is, however, possible, that the formation and disappearance happen so quickly that this structure was not observed.

In the late embryonic development starting in stage 5 (fig. 2j-k), the embryo keeps growing and first pigmentation becomes visible. Also, the eyes become pigmented and the skeletal development starts in the caudal fin (FISCHBACH et al. 2024). Then anal and dorsal fins differentiate and the fin buds of the pectoral fin become visible. Shortly before hatching the embryo exhibits constant movements of the not yet fully differentiated pectoral fins and lastly the embryo even flips within the egg (fig. 2l, Stage 6) until it breaks free. The opercular movement in combination with the presence of skeletal elements of the skull and gills (FISCHBACH et al. 2024) indicate that at the last embryonic stages respiration can already occur via the gills. In this stage, the authors also hypothesize, that the embryos also already possess a functioning mouth, since the yolk sac is fairly well depleted. The authors also hypothesize that the gastrointestinal system is mostly developed, as larvae already possess an anus right after hatch..

The subsequent larval development is described in FISCHBACH et al. (2024) and, together with the present study, provides comprehensive insights into the early life history of garfish, offering a valuable framework for comparative developmental and ecological research. Since garfish occupy diverse ecological niches worldwide, studies on their early life stage tolerance to environmental variability could be aligned to enhance our understanding of their adaptive potential under changing climatic conditions. Moreover, such data may also serve as a foundation for Evo-Devo approaches, enabling investigations into the evolutionary conservation and divergence of developmental processes among related species, and thereby contributing to a broader understanding of phenotypic plasticity and developmental constraints in teleost evolution.

Literature

ALTER, K., & M.A. PECK. 2021. Ocean acidification but not elevated spring warming threatens a European seas predator. Science of The Total Envi-

ronment 782, 146926. https://doi.org/10.1016/j.scitotenv.2021.146926

Boughton, D.A., B.B. Collette, & A.R. McCune. 1991. Heterochrony in jaw morphology of needlefishes (Teleostei: Belonidae). Systematic Biology 40, 329-354. https://doi.org/10.1093/sysbio/40.3.329

Collette, B. 2003. Family Belonidae Bonaparte 1832. California Academy of Sciences Annotated Checklists of Fishes 16, 1-22.

Collette, B. 2003. Order Beloniformes, Belonidae, 1104-1115. In: The living marine resources of the Western Central Atlantic (Carpenter, K., ed.). Rome: American Society of Ichthyologists and Herpenteologists Special Publication.

Collette, B., M. Ge, N. Parin & S. Mito. 1984. Beloniformes: Development and Relationships, In: Ontogeny and systematics of fishes: based on an international symposium dedicated to the memory of Elbert Halvor Ahlstrom (Moser, H., W. Richards, D. Cohen, M. Fahay, A. Kedall Jr., & S. Richardson, eds). Allen Press,Lawrence, USA.

Collette, B., & N. Parin. 1986. Belonidae, 604-609. In: Fishes of the North-eastern Atlantic and the Mediterranean (Whitehead, P., M. Bauchot, J. Hureau, J. Nielsen & E. Tortonese, eds). UNESCO, Paris.

Collette, B.B., K.E. Bemis, N.V. Parin, & I.B. Shakhovskoy. 2018. Order Beloniformes: Needlefishes, Sauries, Halfbeaks, and Flyingfishes: Part 10. Yale University Press, New Haven, Conneticut and London.

Collette, B.B., & K. Cole. 2010. Reproduction and development in epipelagic fishes, 21-63. In: Reproduction and sexuality in marine fishes: patterns and processes (Cole, K.S., ed.). Berkeley: University of California Press.

Collette, B.B., & N. Parin. 1970. Needlefishes (Belonidae) of the Eastern Atlantic-Ocean. Atlantide Report 11, 7-60.

Dorman, J. 1991. Investigations into the biology of the garfish, Belone belone (L.), in Swedish waters. Journal of Fish biology 39, 59-69. https://doi.org/10.1111/j.1095-8649.1991.tb04341.x

Dorman, J.A. 1988. Diet of the garfish, Belone belone (L.), from Courtmacsherry Bay, Ireland. Journal of Fish Biology 33, 339-346. https://doi.org/10.1111/j.1095-8649.1988.tb05476.x

Essner, J.J., J.D. Amack, M.K. Nyholm, E.B. Harris, & H.J. Yost. 2005. Kupffer's vesicle is a ciliated organ of asymmetry in the zebrafish embryo that initiates left-right development of the brain, heart and gut. Development 132, 1247-1260. https://doi.org/10.1242/dev.01663

Fischbach, V., P. Thieme, P. Polte, & T. Moritz. 2024. Delineating early life stages of Belone belone: analysis of external morphology and postcranial skeletal development of the garfish. Canadian Journal of Zoology 102, 934-939. https://doi.org/10.1139/cjz-2024-0004

Fonds, M., H. Rosenthal, & D.F. Alderdice. 1974. Influence of temperature and salinity on embryonic development, larval growth and number of vertebrae of the garfish, Belone belone, 509-525. In: The early life history of fish (Blaxter, J., ed.). Springer Berlin, Heidelberg.

Jiang, R.J., F. Yang, F. Chen, R. Yin, M.Z. Liu, W.B. Zhu, A. Guo, & L.W. Liu. 2022. Assessing trophic interactions among three tuna species in the Solomon Islands based on stomach contents and stable isotopes. Frontiers in Marine Science 9, 1-15. https://doi.org/10.3389/fmars.2022.961990

Korzelecka-Orkisz, A., K. Formicki, A. Winnicki, M. Bonisławska, J. Szulc, M. Biernaczyk, A. Tański, & W. Wawrzyniak. 2005. Peculiarities of egg structure and embryonic development of garfish (Belone belone (L.)). Electronic Journal of Ichthyology 2, 42-53.

Lovejoy, N.R., & B.B. Collette. 2001. Phylogenetic relationships of new world needlefishes (Teleostei: Belonidae) and the biogeography of transitions between marine and freshwater habitats. Copeia 2001, 324-338. https://doi.org/10.1643/0045-8511(2001)001[0324:Pronwn]2.0.Co;2

Lovejoy, N.R., M. Iranpour, & B.B. Collette. 2004. Phylogeny and jaw ontogeny of beloniform fishes. Integrative and Comparative Biology 44, 366-377. https://doi.org/10.1093/icb/44.5.366

Miller, B., & A.W. Kendall. 2009. Early life history of marine fishes. University of California Press, Berkeley and Los Angeles, USA.

Nichols, J., & C. Breder Jr. 1928. About flying fishes. Natural History (New York) 28, 64-77.

Polte, P., & H. Asmus. 2006. Intertidal seagrass beds (Zostera noltii) as spawning grounds for transient fishes in the Wadden Sea. Marine Ecology Progress Series 312, 235-243. https://doi.org/10.3354/meps312235

Rosenthal, H., & M. Fonds. 1973. Biological observations during rearing experiments with the garfish Belone belone. Marine Biology 21, 203-218. https://doi.org/10.1007/BF00355251

Schlesinger, G. 1909. Zur Phylogenie und Ethologie der Scombresociden. Verhandlungen der Kaiserlich-Königlichen Zoologisch-Botanischen Gesellschaft 59, 302-339.

Seifert, R. 1938. Die Bodenfauna des Greifswalder Boddens. Ein Beitrag zur Ökologie der Brack-

wasserfauna. Zeitschrift für Morphologie und Ökologie der Tiere 34, 221-271.

VON WESTERNHAGEN, H. 1974. Incubation of garpike eggs (*Belone belone* Linné) under controlled temperature and salinity conditions. Journal of the Marine Biological Association of the United Kingdom 54, 625-634. https://doi.org/10.1017/S0025315400022797