

Haemoglobin and size dependent constraints on swimbladder inflation in fish larvae

Hämoglobin und gröÙebedingte Beschränkungen der Schwimmblasenbefüllung bei Fischlarven

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Summary: In developmental studies of fish species (especially physostomians) it could be demonstrated, that the lack of haemoglobin during larval and juvenile stages is a relatively common phenomenon. Generally it is linked with body translucency. In representatives of the families Galaxiidae, Osmeridae and Clupeidae, partly reared, partly observed immediately after being caught in the wild, it turned out, that this condition coincides with a considerable delay in swimbladder inflation. To determine the moment of its first inflation, larvae placed in a hermetic chamber were observed under a dissecting microscope. While lowering the pressure, the expanding swimbladder showed whether or not its content is really gaseous. The reason postulated to be responsible for the delayed inflation is that larvae lacking haemoglobin do not have the possibility of oxygen transport to their buoyancy organ by means of the blood. Apart of this, capillarity force calculations and body force estimations show that with decreasing size the constraints linked with surface tension increase overproportionally. While in larger sized larvae like trout we could demonstrate inflation by swallowing air, in species with small larvae this was not the case. Below a certain size, even in physostomians, the ductus pneumaticus is no alternative to the blood pathway for swimbladder inflation.

Keywords: swimbladder, haemoglobin, surface tension, fish larvae, translucency syndrome, white-bait stage, critical life stages

Zusammenfassung: Bei Untersuchungen der Larvalentwicklung von (hauptsächlich physostomen) Fischen fiel auf, dass es zahlreiche Arten mit hämoglobinlosen Larven- und Jugendstadien gibt, was oft mit stark durchscheinendem Aussehen verbunden ist. Bei Vertretern der Familien Galaxiidae, Osmeridae und Clupeidae konnte durch Beobachtung von aufgezogenen oder frisch gefangenen Larven gezeigt werden, dass dieser Umstand mit einer erheblichen Verspätung der Gasbefüllung der Schwimmblase

einhergeht. Durch Beobachtung in einer hermetischen Kammer unter einem Präparier-Binokular, konnte bei Herabsetzung des Druckes durch die entsprechende Ausdehnung der Schwimmblase die Gas-Natur des Schwimmblaseninhaltes und der Zeitpunkt seines Erscheinens ermittelt werden. Die Verspätung der Gasbefüllung der Schwimmblase dürfte an der Unfähigkeit liegen, in Ermangelung von Hämoglobin über das Blut Sauerstoff zum Auftriebsorgan zu führen. Berechnungen der Kapillarkräfte und Abschätzungen der Körperkräfte zeigten, dass durch Oberflächenspannung bedingte Kräfte mit abnehmender Größe überproportional ansteigen, und sich der Überleitung von Luft über den Ductus pneumaticus widersetzen. Dies ist bei größeren Fischlarven, wie wir bei Salmoniden nachweisen konnten, nicht der Fall. Selbst bei Physostomiern ist der Ductus pneumaticus, wenn es sich um kleine Larven handelt, keine Alternative zur Blutbahn für die Gasbefüllung der Schwimmblase.

Schlüsselwörter: Schwimmblase, Hämoglobin, Oberflächenspannung, Fischlarven, Durchsichtigkeitsyndrom, durchscheinende Jungfische, kritische Lebensstadien

Resumen: En estudios sobre el desarrollo larvario de peces (especialmente fisóstomos) pudimos observar que hay numerosas especies con larvas y juveniles carentes de hemoglobina, lo que va acompañado de un aspecto altamente translucente. En representantes de las familias Galaxiidae, Osmeridae y Clupeidae, tanto criados como recién capturados, se pudo constatar que esto coincide con un apreciable atraso en el acto de inflar su vejiga natatoria. La presencia de gas en la vejiga natatoria, como su punto de aparición durante el desarrollo, pudieron ser demostrados en una cámara hermética, observando por un microscopio de disección la dilatación al hacer bajar la presión. Sostenemos que la razón del atraso es, que a falta de hemoglobina son incapaces de transportar oxígeno por vía sanguínea hacia su órgano hidrostático. Cálculos de capilaridad como estimaciones de la fuerza corporal demuestran que con una disminución del tamaño las fuerzas de tensión superficial aumentan sobreproporcionalmente. Larvas pequeñas, y la gran mayoría lo son, no pueden vencer la tensión superficial para llenar la vejiga natatoria tragando aire, como lo hacen las relativamente grandes larvas de Salmonidae. En larvas pequeñas, frente al llenamiento por vía sanguínea, incluso tratándose de fisóstomos, el ductus pneumaticus no es alternativa para inflar la vejiga natatoria.

Palabras claves: Vejiga natatoria, hemoglobina, tensión superficial, larvas de peces, síndrome de translucencia, puyes cristalinos, etapa crítica de desarrollo

1. Introduction

1.1. Observations leading to this study

The finding that some Galaxiid juveniles have no red blood up to a length of 6 cm (Busse 1993) was the initial spark, which lastly led us to do this study. Observing diverse developmental stages of some representatives of the family Galaxiidae a striking phenomenon became evident: The almost transparent and scarcely pigmented *Galaxias maculatus* larvae which lack red blood are widely retarded in their ability to achieve neutral buoyancy, while the red-blooded *Brachygalaxias gothei* larvae soon after hatching are able to inflate their swimbladder and become buoyant. We observed

larvae available of some other groups such as Osmeridae and Clupeidae, which also have transparent larval or juvenile stages, realizing a similar delay of buoyancy swimming. All seem to have constraints in their ability for their initial filling of the swimbladder with gas. We suspected a close relationship between haemoglobin and swimbladder inflation, even though they are physostomian fishes, which commonly are thought to inflate it by gulping air and conducting it via their ductus pneumaticus.

1.2. A gap in knowledge and possible reasons

Physiological mechanisms of the swimbladder inflation and regulation of adult or at

least of middle sized fish are fairly well known (for overviews and literature see Bone et al. 1995, Penzlin 1996, Pelster 1998). This is not the case for the early developmental stages, especially of species with tiny larvae. The literature related to ontogeny is scattered in diverse kinds of publications ranging from fishery journals to physiological and even medical ones. There are many descriptive studies for staging the development (Moser et al. 1984, Copp et al. 1999, see there for more literature). The goals reach from species identification helps of larvae in the wild, aquaculture aims to highly specialized laboratory research on developmental determinants, sometimes even with transgenic larvae of a few laboratory model species like the Zebrafish *Danio rerio* (see “The Zebrafish Information Network [ZFIN]” consulted 2006). There is a gap in knowledge just in the middle between those extremes especially respecting haemoglobin appearance and swimbladder inflation. This might have the following reasons:

- a) In studies for staging the development or the guides for species identification of larvae, (e. g. Smith 1989) commonly only preserved specimens are examined. Such standard methods were used in the majority of the contributions to the book of Moser et al. (1984). This book is an overview and guide, but also shows that swimbladder and blood condition in most developmental studies rarely were considered. These features remain undetected because neither a bubble of air indicating swimbladder presence, nor the blood circulation in the vessels persist in preserved larvae. The gas usually dissolves within one or few days after fixation, stationary erythrocytes are difficult to see, the blood colour fades and the tissues become opaque.
- b) While there are many species with red-blooded larvae which are easy to rear, the ones with no haemoglobin according to

our experience are difficult if not impossible to raise. This has hindered comparative studies between both larval types.

- c) Physical parameters other than viscosity commonly have not been the focus of most developmental studies. This is especially important, particularly since small organisms reach or surpass critical values, intuitively not expected. This applies for surface tension and solubility dynamics of gases.
- d) The availability of living larvae has been a considerable obstacle, because many species which are difficult to breed have to be collected in the wild and observed immediately, to document blood and swimbladder condition at different ontogenetic stages.

In spite of this there are some publications encouraging us to continue. Pelster and Burggren (1996) provided a proof that there is a close relationship between first swimbladder inflation and the presence of haemoglobin in zebrafish (*Danio rerio*) artificially deprived of haemoglobin. As a counterpart to this experiment, many fish larvae naturally lacking haemoglobin exist in the wild. The aim of the present study is to give some examples of this phenomenon and a timetable of the swimbladder inflation in relation to the appearance of haemoglobin in different fishes. While remaining primarily descriptive, we also consider physical measurements and estimations, in order to piece together a rough review of the diversity of ways of initial inflation mechanisms of the swimbladder in teleosts.

2. Materials and methods

The study objects were larvae and juveniles of some Galaxiidae, Osmeridae, Salmonidae, Cyprinidae and Clupeidae. Depending on their availability they were obtained by rearing the larvae from ova coming from spontaneous spawning in aquaria, from striping wild caught or aquaculture adults.

Tab. 1: Species involved in this study, observations and experiments done, as well as additional hints to other sources of information used.

Tab. 1: Arten, die in diese Untersuchung einbezogen wurden, getätigte Beobachtungen und Versuche, sowie zusätzlich verwendete Informations-Quellen.

taxon	developmental observations	timing of Hb appearing	timing of 1st swim bladder inflation	air gulping observed/ grid experiment	pressure experiment	heart volume estimation (histology)	haematological profile	remarks and sources
<i>Galaxias maculatus</i>	yes	yes	yes	could not be clearly observed	yes: lowering, raising	yes	yes	developmental data partly: Campos (1970); heart histology: Busse and Campos (1996); haematological profile: Jaramillo (2005)
<i>Galaxias brevipinnis</i>	some	yes	no data	no data	no	yes	no	preserved specimens and information of Hb appearing provided by McDowall
<i>Galaxias fasciatus</i>	some	yes	no data	no data	no	no	no	preserved specimens and information of Hb appearing provided by McDowall
<i>Galaxias zebratus</i>	yes	yes	yes	no data	no	no	no	data drawn from photographs by Cambray (1998), and Cambray (pers. comm.)
<i>Brachygalaxias bullocki</i>	yes	no	no own data	no data	no	no	no	data according Campos (1972)
<i>Brachygalaxias gothei</i>	yes	yes	yes	not observable	yes in larvae: lowering, raising	observations but no histology	no	mainly this study
<i>Osmerus eperlanus</i>	yes	yes	yes	not observable	yes, in larvae and juveniles lowering, raising	observations but no histology	no	this study; early developmental data, see Lillelund (1961)
<i>Alosa alosa</i>	yes	yes	yes	not observed	yes, in finray juvenile	no	no	this study; see also Beeck et al. (2006)
<i>Clupea harengus</i>	yes	yes	yes	yes, observed	no	no	no	this study; see also Ueberschär (2006)
<i>Sardina pilchardus</i>	yes	no	yes	no	no	no	no	see also Ueberschär (1995; 2006)
<i>Oncorhynchus mykiss</i>	yes	yes	yes	yes, grid experiment	yes, in larvae and juveniles: lowering, raising	no own data	no own data	Developmental data: Picard and Voßwinkel (1996); others: mainly this study
<i>Danio rerio</i>	yes	yes	yes	not observable	yes, in larvae, juveniles and adults: lowering, raising	not done in our study	not done in our study	data from literature, see ZFIN (cons. 2006), Pelster and Burggren (1996), and complemented with own data
<i>Pseudorasbora parva</i>	no	no	no	no	yes in half grown individuals lowering, raising	not in our study	no	mainly this study

Due to sometimes enormous mortality and difficulties to rear, especially in the case of smelts *Osmerus eperlanus*, reared stocks had to be complemented with larvae or juveniles taken directly from the wild for immediate examination. The culture of larvae was sometimes easy when it could be accomplished feeding them by conventional methods using infusoria cultures, or by keeping them in aquaria where they fed on the naturally existing small plankton organisms or the ones they took from the aufwuchs. For the translucent pelagic larvae culture proved to be difficult. Especially smelt larvae refused to feed and starved. Therefore it was a great advance when they accepted rotifers, *Brachionus plicatilis*, from a culture fed on algae (planktonic Chlorophyceae: *Nannochloropsis* sp.). Even with this method smelts could not be raised to more than a size of 15 mm. Part of our *Galaxias maculatus* were raised in Chile in a similar way as the smelts according to methods described by Dantagnan et al. (1995) and provide part of our data.

The observations were made on live material, when necessary anesthetized with ethyl 3-aminobenzoate methanesulfonate (Tricaine, Fluka), on freshly killed individuals or on freshly fixed ones. Also formalin

or alcohol preserved collection specimens were used for comparison, but the problem of the disappearance of the swimbladder's gas reduces their suitability for our special aim. Photographs proved to be a better approach than preservation. Also histological sections previously used for other studies served as complementary comparative material in some cases.

The difference in the index of refraction for gases in relation to water or translucent body tissues is the reason that an inflated swimbladder is easily visible. Also the otic capsules have parts which might be confused with air bubbles. They really look different, but as an additional proof of true gas-filled inner cavities we placed the larvae into a simple transparent vacuum chamber made of a cavity block stage (fig.1). Lowering the pressure, the expansion of the gas can be observed under a dissecting microscope. The volume increase of the swimbladder forces the larva to rise like a Cartesian diver. This is easier to observe with an anesthetized larva, to avoid active compensatory swimming movements. Especially with a further lowering of the pressure under certain circumstances it is possible to demonstrate the opening of the ductus pneumaticus (fig. 4). Another device

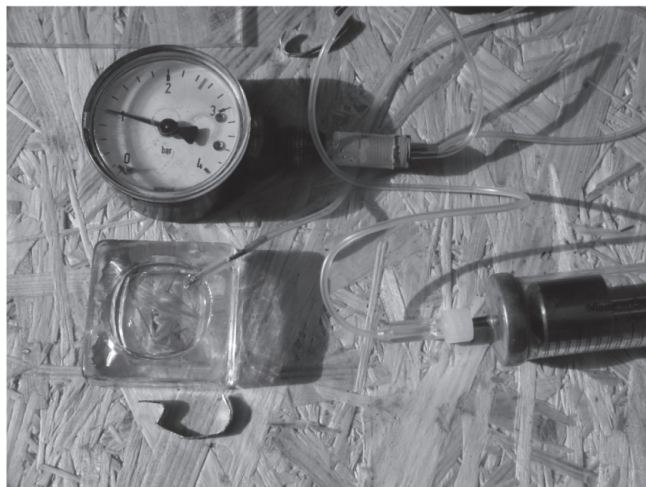


Fig. 1: Block stage for microscopy which can be hermetically sealed with a plane glass. Connected with a syringe it is possible to observe under a dissecting microscope a larva's swimbladder by lowering the pressure with the syringe.

Abb. 1: Mit einem Planglas verschließbares Blockschälchen, um die Schwimmblase einer Fischlarve unter dem Binokular zu beobachten, während mit Hilfe einer Injektionsspritze der Druck gemindert wird.

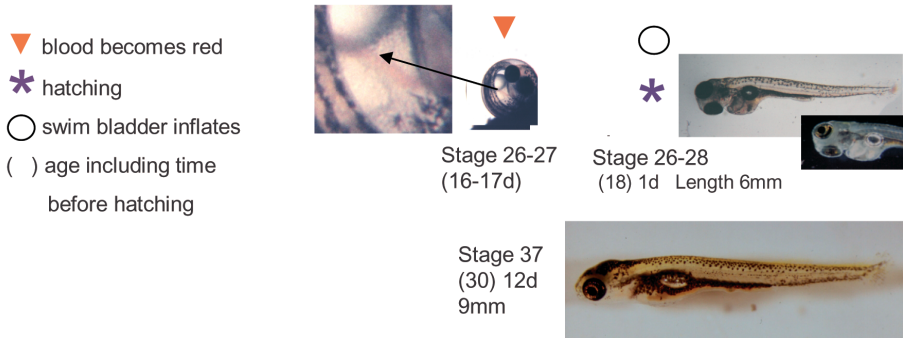


Fig. 2: Developmental details of *Brachygalaxias gothei*.
Fig. 2: Details aus der Entwicklung von *Brachygalaxias gothei*.

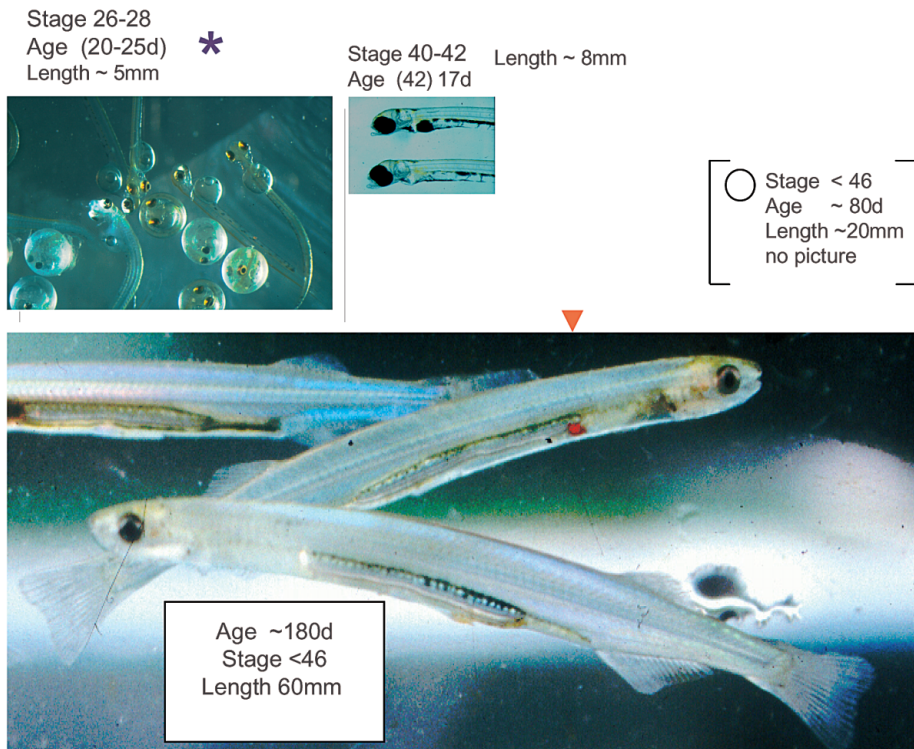


Fig. 3: Developmental details of *Galaxias maculatus* (symbols as in fig. 2).
Abb. 3: Details aus der Entwicklung von *Galaxias maculatus* (Symbole wie in Fig. 2).

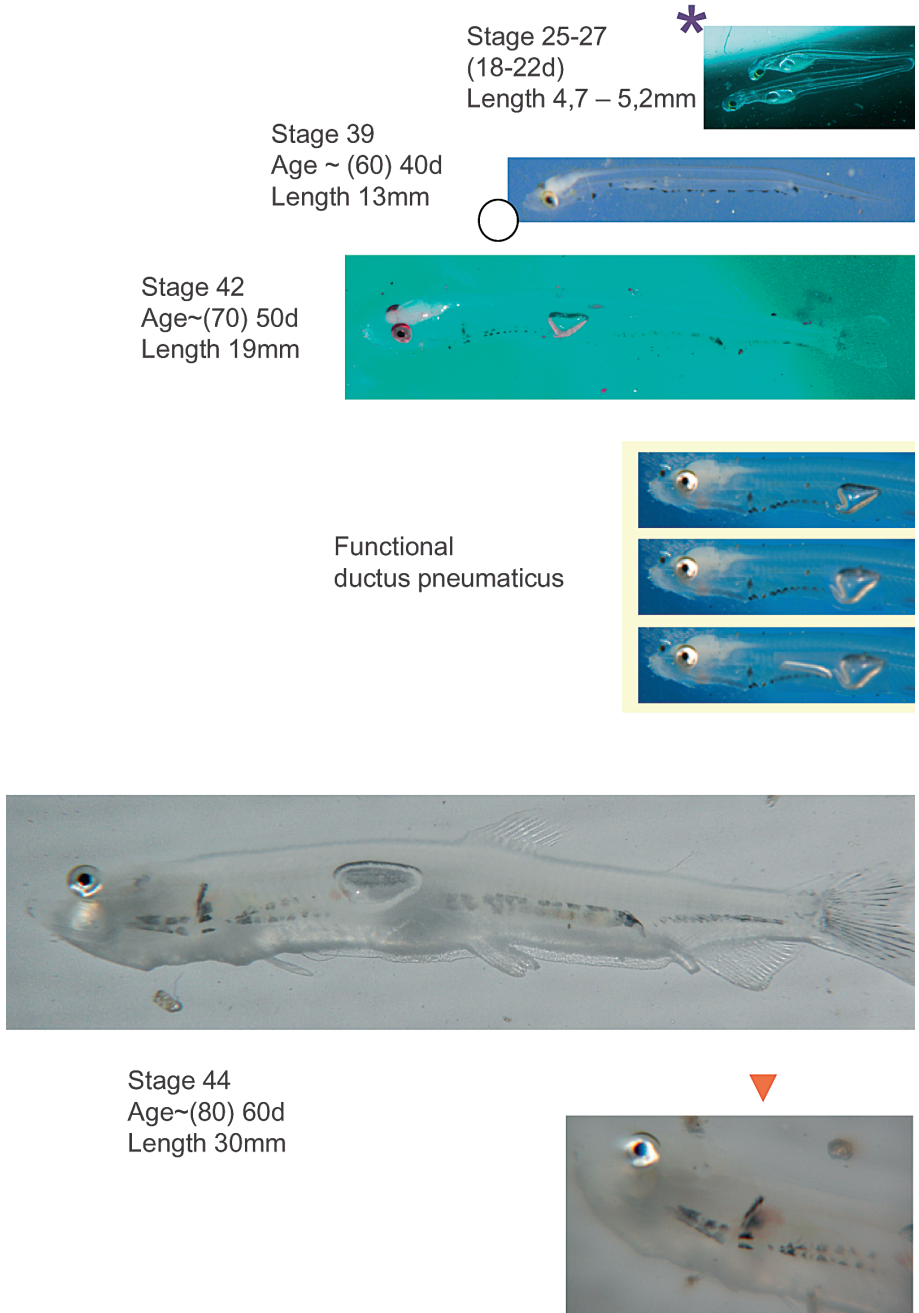


Fig. 4: Developmental details of *Osmerus eperlanus* including a demonstration of the functionality of the *ductus pneumaticus* by lowering of the pressure (symbols as in fig. 2).

Abb. 4: Details aus der Entwicklung von *Osmerus eperlanus*; Nachweis der Durchgängigkeit des *Ductus pneumaticus* durch Druckminderungs-Versuch (Symbole wie in Fig. 2).

used was a pressure chamber: a bottle with a manometer and water with a fish inside inflated with a manual air pump.

An experimental device used for trout larvae consisted of a small aquarium with a continuous water flow circulating from a 50 l tank, where water was kept between 10 and 16 °C. It had two compartments, one covered with a grid of a mesh of 12 mm directly under the surface, the other with a free water surface (fig. 6). For the other smaller fish there was the problem that the mesh would have to be smaller, which had the inconvenience to retain air bubbles (a result of surface tension) which would have biased the experiment.

Although homologizing of stages is not really possible between different species we used the staging numerals of Picard and Voßwinkel (1996) for the rainbow trout. For the larger units of life history, Kendall et al. (1984) gave a review of the terminology of diverse authors. Considering the heterogeneity of developmental stages, the terminology proposed by Balon (1999) was not entirely applicable. We had to restrict to the terms: hatching, larva (in an inclusive sense with “free embryo”), notochord (=tail) flexion, finray juvenile.

The first and principal method of this study was direct observation, enhanced by the use of dissecting microscopes, digital and video cameras with macro lenses. The second was the calculation and/or estimation of physical parameters.

3. Results

3.1. Ontogenetic appearing of haemoglobin and swimbladder inflation

As Pelster and Burggren (1996, see introduction) showed for zebrafish larvae (*Danio rerio*) that swimbladder inflation was impaired by ablation of the haemoglobin by phenylhydrazine or by delayed red blood cell formation in hyperoxia, we show as a general

result the relation in time between haemoglobin appearance and swimbladder inflation in the ontogeny of some selected fish in a descriptive approximation. It is widely retarded in species in which larvae (and juveniles) naturally lack haemoglobin. Fishes in which the relationship between presence of haemoglobin and the ontogenetic timing of swimbladder inflation was especially conspicuous were found in the Galaxiidae. Thus this family is of particular interest because it demonstrates two very different developmental modalities within the same group.

3.1.1. Some examples of Galaxiidae

For *Brachygalaxias bullocki*, Campos (1972, p. 539) describes a developmental stage in which he could “clearly distinguish a vitelline circulation”. This occurs already before hatching. After hatching (at the size of 5,6-5,9 mm) he mentions the visibility of the swimbladder. We could confirm the same for *Brachygalaxias gothei* (fig. 2), but additionally it is necessary to remark that a rosy blood colour is clearly visible and the movement of the erythrocytes through the ductus cuvieri along the surface of the yolk sac becomes especially evident. Further we point out that the tiny bubble of the swimbladder visible in the abdominal hole dorsally to the yolk is really filled with gas. The proof is that it expands when pressure is lowered forcing the larva to rise like a Cartesian diver. Apart from these experimental conditions, under normal pressure soon after hatching the larva achieves buoyancy. The *Brachygalaxias* larva has a normal increasing pigmentation with melanophores and after development of the fin rays becomes a juvenile very similar to adults, and undergoes no further metamorphosis. *Galaxias zebratus* from South Africa follows a similar developmental scheme (see photographs in Cambrey 1998).

In *Galaxias maculatus* the larvae are almost unpigmented (fig. 3). Campos (1970) com-

mented that he could not see the vitelline circulation in the embryos or larvae (hatching size about 5,0 mm), but did not recognize that this is due to the lack of haemoglobin-bearing erythrocytes. According to our observations no red blood is visible, nor is a rosy sheen present on the gills, heart, ductus cuvieri or other vessels. No swimbladder is detectable. The larva submitted to pressure variations does not respond like a Cartesian diver. In open water it sinks continuously, and compensates by repeated bursts of active struggling.

Several *Galaxias* species are known to grow for months, maintaining a translucent developmental condition, in some cases up to 10 times the size of hatching: the slender whitebait stage (McDowall 1970, 1984). In *Galaxias maculatus* the whitebait stage can last up to 6 months and a length of 6 cm: a time span during which they have no haemoglobin and develop a heart hypertrophy to compensate this deficiency (Busse 1993, Busse and Campos 1996).

Although we have no exact figures, when *G. maculatus* inflates its swimbladder becoming buoyant, this occurs much later than in *Brachygalaxias*, but still before the end of the whitebait stage. Tentatively this is at a length of 15 mm (as a finray juvenile) and an age of about two months: long before the blood becomes red. Lack of haemoglobin delays but does not preclude swimbladder inflation.

G. maculatus remains translucent, as larva and whitebait, for up to six months (figs. 3, 7). Heart, gills and liver are not red. The spleen is the first organ to show red colouration. Then, a kind of secondary metamorphosis occurs: the fish shrinks, becomes pigmented and red blooded, takes on adult shape and begins to grow again. Recent haematological studies (Jaramillo 2005) confirmed the lack of mature erythrocytes until the end of the whitebait stage.

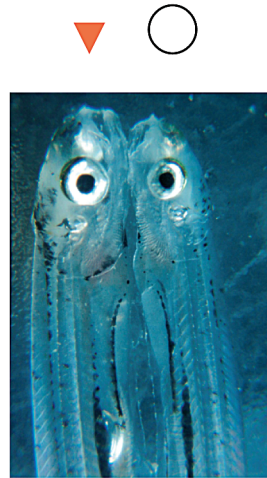
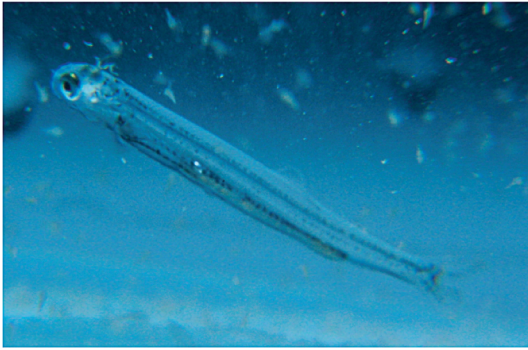
3.1.2. Smelt, an example of the Osmeridae

The closest relative of Galaxiids in Germany is the smelt, *Osmerus eperlanus* (fig. 4). Developmental descriptions (Ehrenbaum 1894, Lillelund 1961) are incomplete, especially concerning larval and post larval stages. No information on blood and swimbladder inflation is available, probably due to the enormous difficulty in rearing the fish. In this species we found a developmental scheme like that of *Galaxias maculatus* at least for the first three months of life. Similarly, near hatching (with 5 mm) it is difficult to see blood circulation, due to the scarcity of blood cells. The swimbladder fills with gas long after hatching and at a length of 13 to 19 mm. We were not able to demonstrate if this swimbladder is filled by gulping air, but it does occur before there are any signs of blood colouration. We could also demonstrate, by lowering the atmospheric pressure, that the ductus pneumaticus permits the passage of gas to the intestine (fig. 4). Blood becomes red when the fish is at least two months old and reaches 30 mm in length (as a juvenile with complete fin rays). This happens at a smaller size than in *G. maculatus*, and the transformations are not as dramatic as in them. Body shrinkage could not be observed. It has not been studied whether or not the heart becomes smaller, as in *G. maculatus*. The first organs to become red are the spleen, the liver and the gills.

For a considerable part of its non-red-blooded existence, the smelt lacks an inflated swimbladder and must continuously struggle to avoid sinking. They are even more difficult to maintain in captivity than *G. maculatus*. Their food requirement appeared to be great: in the Elbe River we caught them among very dense plankton clouds.

3.1.3. Some Clupeidae

The only clupeid larvae we could study in living condition were shad and herring. Shad



Stage 42- 44
Age (~50) 28 d
Length 21mm

Fig. 5: Developmental stage of *Alosa alosa*: swim bladder inflation coincides with the appearing of red blood (symbols as in fig. 2).

Abb. 5: Entwicklungsstadium von *Alosa alosa*: Schwimmblasenbefüllung fällt zeitlich mit dem Rotwerden des Blutes zusammen (Symbole wie in Fig. 2).

▼ Stage 21
Age (12d)
Length~21mm
no picture



* Stage 33
Age (33 d)
Length~25mm



Stage 40-42
Approx. (50) 20d
Length 30mm

Fig. 6: Developmental details of *Oncorhynchus mykiss* (symbols as in fig. 2); the individual on the left part of the tank was hindered to surface by a grid on the top.

Abb. 6: Details aus der Entwicklung von *Oncorhynchus mykiss*: (Symbole wie in Fig. 2); das Individuum auf der linken Seite des Behälters wurde durch ein Gitter am Luftholen gehindert.

Fig. 7: Observed growth curve fragments of fish larvae of different species and timing when the different events occur. All larvae smaller than the size indicated by the arrow are assumed to inflate their swim bladder by a blood circulatory pathway if they have haemoglobin (the only example for this condition shown here is *Brachygalaxias gothei*). Haemoglobinless larvae would have to wait until they grow to inflate it mechanically via the ductus pneumaticus, or by other less effective circulatory pathways, and/or direct gas liberation (see 5.5.).

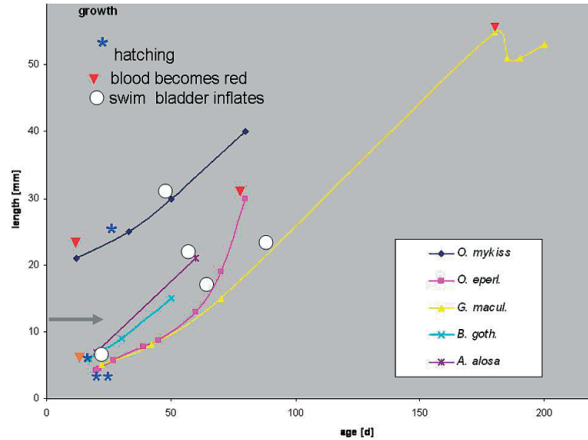


Abb. 7: Ermittelte Wachstumskurven-Abschnitte von Larven verschiedener Arten und Auftreten verschiedener Ereignisse. Für alle Larven, deren Größe unter der Pfeilmarke liegt, wird angenommen, dass sie ihre Schwimmblase auf dem Wege der Blutbahn füllen müssen, sofern sie Hämoglobin haben (einziges aufgeführtes Beispiel ist *Brachygalaxias gothei*). Hämoglobinlose Larven müssten entweder warten, bis sie wachsen, und es mechanisch über den Ductus pneumaticus schaffen, oder aber weniger effektive Blutbahnwege dazu nutzen einschließlich einer Freisetzung von Gas vor Ort (siehe 5.5.)

Fig. 8: Relationship between capillary force and body force in accordance to the size. One curve is the capillary ascent of water in a glass capillary depending on its inner diameter, the other is a relative value of how the force of an organism is related with the size (assuming that it increases with the cross section of any muscle). The point where both curves cross each other was chosen arbitrarily, in a position nearly corresponding to the size conditions of a trout which anyway permit the larva to gulp air from the surface. While in the area on the right hand side one can appreciate that the constraint due to capillary force becomes less critical and even negligible, to the left in contrast, rapidly it becomes immense with decreasing size.

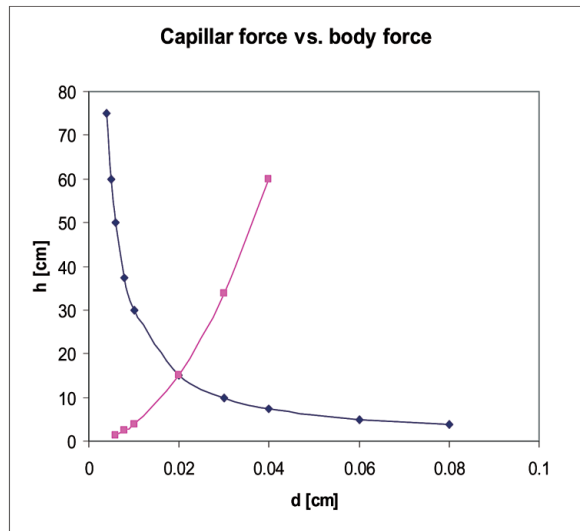


Abb. 8: Verhältnis zwischen Kapillarkraft und Körperkraft im Zusammenhang mit der Größe. Eine Kurve zeigt den Anstieg von Wasser in einer Glaskapillare bezogen auf ihren Innendurchmesser, die andere ist ein relativer Wert, wie die Körperkraft mit der Größe variiert, unter der Annahme, dass diese vom Querschnitt eines jeden Muskels abhängt. Der Kreuzungspunkt wurde willkürlich gewählt bei einer Größe wie der einer Forellenlarve, die nachgewiesenermaßen ausreicht, um durch Luftschlucken die Schwimmblase aufzufüllen. Die Fläche rechts davon zeigt, dass die Behinderung durch Kapillarkraft abnimmt und bald vernachlässigbar wird, links jedoch mit der Größenabnahme immens ansteigt.

(*Alosa alosa*) (fig. 5), herring (*Clupea baren-gus*) and sardine (*Sardina pilchardus*). They have a roughly similar larval appearance to *Galaxias maculatus* and smelts. The shad larva is larger, but all are very scarcely pigmented and lack red blood. They also have to swim actively against sinking: we could not detect a swimbladder at early stages, even using the Cartesian diver device.

The first inflation of the swimbladder in the shad was documented in a fin ray juvenile at a length of 21 mm and an age of about two months (fig. 5). We preferred not to make further pressure experiments to avoid increasing the already high mortality. Swimbladder inflation coincided with the time when the gills began to show a rosy sheen. This is intriguing, because in a fish with no rete mirabile in the swimbladder, one would not expect any correlation (see discussion). We could not observe gulping of air although the large size of the juvenile makes this mechanism plausible (see trout and section of surface tension).

The first swimbladder filling in the herring was observed at the end of the larval stage, close to metamorphosis. Herring larvae reared under laboratory conditions showed first swimbladder inflation around 40 days after hatching, when they reached a total length of about 25 mm (temperature 12.17 °C; salinity 16 psu [about 16 ‰]) at the same time the gills became pinkish. Under laboratory conditions, herring larvae tend to swallow small gas bubbles in early life stages just after commencement of feeding. It is not yet proven if they confuse the bubbles with food or if this behaviour targets a first filling of the swimbladder. They attack the bubbles, showing the typical s-shape as common when attacking a prey. The bubbles accumulate in the gut, causing irregular buoyancy, and the larvae drift on the surface. The mortality rate is high in this stage.

Larvae of *S. pilchardus*, caught in larvae surveys in the Atlantic, showed periodic swim-

bladder inflation during the night at a size of 10-15 mm (Ueberschär 1995). They are much younger in this stage than are herring larvae mentioned above. However, in contrast to the herring larvae, they showed no signs of appearance of haemoglobin in this stage.

3.1.4. Trout

Rainbow trout (*Oncorhynchus mykiss*) have a quite different development (fig. 6). Larvae are large and they are benthic for a long part of their development. The blood becomes red before hatching; heart, ductus cuvieri and other blood vessels look intensely red and the erythrocytes can clearly be seen moving through the vessels. After hatching larvae lie on the bottom most of the time. The swimbladder is not inflated. In this condition they already begin to eat *Daphnia* sp.

In our two groups of trout hatchlings of the same age, the ones which were permitted to come to the surface approx. 20 days after hatching (Fig. 6) came up to gulp air, and after some attempts they became more or less neutrally buoyant. The others, which were prevented from reaching the surface by a grid, could not inflate their swimbladders. They made repeated attempts to surface. When all specimens of the first group were swimming, we removed the grid from the second one. They surfaced vigorously, swallowed air, and were all buoyant within the next 15 minutes, just like their siblings.

3.2. Physical aspects

3.2.1. Size and surface/volume relationship

The finding of Pelster and Burggren (1996) that ablation of haemoglobin does not affect oxygen consumption in *Danio rerio* is essential to understand that the lack of this gas transport pigment is not critical for the oxygen supply of tissues in such small organisms. This might be also valid for the

early larvae of *Galaxias maculatus* at similarly small stages, but no longer applies as the fish grows. As in this species, a tenfold increase in length implies a thousandfold increase in volume, and a less favourable surface to volume ratio. The massive heart hypertrophy of *G. maculatus* whitebaits is considered as a compensatory mechanism to the lack of haemoglobins (Busse and Campos 1996). Another indication which confirms this size relationship is that small larvae, regardless if red blooded or not, when anesthetized with tricaine recovered even after some minutes of cardiac failure, while larger ones and juveniles or adults rapidly die.

3.2.2. Size and surface tension

Trout were able to inflate their swimbladders by swallowing air at Picard and Voßwinkel (1996) stage of about 40-42. In our experimental device with a grid preventing surfacing (fig. 6) we tested only rainbow trout (*Oncorhynchus mykiss*), but other salmonids are similar.

When exposed to a pressure of 2 to 4 bar their buoyancy was reduced, but recovered when they gulped air. When the pressure was returned to atmospheric, not all were able to deflate again. This demonstrates that the ductus pneumaticus is functional, at least to inflate the swimbladder and with some restrictions to deflate it. A similar result was shown by subadult individuals of *Pseudorasbora parva* and zebrafish of a comparable size as the trout larvae.

In larvae less than about 10 mm total length we were not able to observe swallowing air. According to Pelster and Burggren (1996) zebrafish larvae do not gulp air but inflate their swimbladder by means of their circulatory system. Although cyprinids are considered to be physostomians, the larvae of zebrafish are functionally physoclistic (=as if the ductus pneumaticus would not exist). An explanation is that such tiny fish larvae

are unable to swallow air because of surface tension, i.e., high capillary forces in the ductus pneumaticus (including mouth, swimbladder itself, etc.).

Ascent in a capillary tube depends on several parameters of the involved fluids and the inner capillary walls and lastly on gravity force. In the special case of water in a glass capillary under standard gravitational conditions, all values are constant. If we vary only the inner diameter (d [cm])

the capillary ascent (h [cm]) is:

$$h = k \frac{1}{d}$$

for glass and water $k \approx 0.3$

$$h \text{ [cm]} \approx \frac{0.3 \text{ [cm}^2\text{]}}{d \text{ [cm]}}$$

The value of k will also vary with the physical properties of the tube surface of the inner walls of ductus pneumaticus, mouth, and other factors.

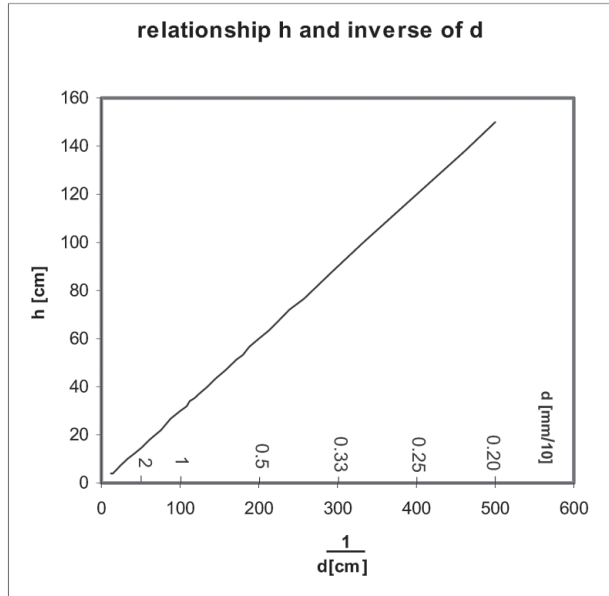
Especially surfactants may lower it. The absolute values of the capillary ascent (fig. 8) are fictive, but the shape of the curve remains the same. The height of the water column in a capillary tube varies as a function of its diameter. Plotting the height values against the inverse of the diameter the relation becomes linear (fig. 9).

If we assume a similar shape in all body parts the diameter of the ductus pneumaticus of a newly hatched *Brachygalaxias gotbei*, *Galaxias maculatus*, *Osmerus eperlanus* or *Danio rerio* it should have about one fifth or one sixth of the diameter of the ductus of an *Oncorhynchus mykiss*.

Assuming that the ductus pneumaticus of a larval trout *O. mykiss* has a diameter of about 0.2 mm, the fish would have to overcome a capillary pressure of 15 cm water column in order to inflate the swimbladder. The small larvae mentioned above (having one fifth of the linear dimensions of a trout larva) would face a pressure of a 70

Fig. 9: Replacing the capillary diameters of the former graph by its inverse values, the ascent curve becomes linear. This allows direct reading of capillary ascent for different sizes. The effort a fish larva has to do is related to those values, but lastly might be lowered by surfactants.

Abb. 9: Bei Ersetzen der Kapillardurchmesser der vorigen Grafik durch ihren Kehrwert wird die Relation geradlinig. Dies erlaubt direktes Ablesen des Anstiegs für verschiedene Größen. Die Kraft, die eine Fischlarve zum Luftholen braucht, steht damit im Zusammenhang, kann aber durch oberflächenaktive Substanzen herabgesetzt werden



cm water column (see fig. 9); the really tiny zebrafish considerably more. The presence of surfactants (Daniels and Skinner 1994) may greatly reduce the magnitude of these figures, but the shape of the relationship remains the same: the overproportional increase of capillary forces with respect to decreasing of size persists (fig. 8).

Only in some larger fish larvae we were able to observe the air gulping action, which might have been the primitive mechanism of swimbladder or lung filling in ancestral fish (Perry et al. 2001). *Polypterus* sp. begins to gulp air at a size of more than 2 cm. It becomes able to use its “lung” as buoyancy organ at a considerable larger size (Bartsch and Britz 1996). To some extent the case is comparable to salamander larvae and tadpoles.

3.2.3. From surfactants to water repellent surfaces

Insects widely make use of water repellent surfaces. Mosquito larvae (Culicidae) cling to the surface by the hydrophobic

border and inner walls of their siphon. This counteracts capillarity, permitting the air to enter the tracheal system. At least in fish larvae there is no evidence for a similar mechanism for the entrance of gas through mouth and ductus pneumaticus. In water repellent surfaces there is a discontinuous transition between the moistened and the dry condition. In consequence if fish larvae would have water repellent inner walls of mouth and air pathways, there would be the risk to stick to the surface in the moment of coming in contact with it. This could be a reason, why the option of water repellent surfaces has not been followed in the same way as in insects. Surfactants in inner cavities are frequent in vertebrates. They would potentially cause a similar effect to fish larvae to cling to the surface during filling of the swimbladder, additionally, they would tend to disperse on the water surface. Although the exact mechanisms are not known, qualitative and quantitative differences in surfactant properties could have evolved, which minimize the risk of the larvae to stick to the water surface.

3.2.4. Lipids versus gases as buoyancy means

Pelster (1997) stated that in pelagic fish larvae the oil droplets in the yolk sac may serve for buoyancy. In smelts this mechanism is not sufficient: they sink relatively fast during the intervals between struggling. Since the density of naturally occurring oils is around 0.9, the oil drop would have to be about ten times the volume of a corresponding gas-filled swimbladder in order to provide a comparable buoyancy effect. This is clearly not the case in galaxiids, smelts, herrings and in many other fish larvae.

5. Discussion

5.1. Comparison with further taxa

For the leptocephali (willow leaf larvae) of the eel (*Anguilla anguilla*), in spite of difficulties of maintaining them in the laboratory, numerous studies exist (i. e. Lecomte-Finiger et al. 2004). Also for other species within the order Anguilliformes Lecomte-Finiger et al. (2004) give information on leptocephali and provide an extensive literature. Nevertheless information about the early blood and swimbladder condition is scarce. Indications about the lack of erythrocytes and haemoglobin are given by Hulet (1978, 124) for the larva of *Ariosoma balearicum* (Family Congridae). Castle (1984) refers to the same feature extending it to other Anguilliform leptocephali. Photographs of live individuals available to us show no swimbladder through the almost transparent body wall (see also Pelster 2004). Lack of haemoglobin linked with lack of an inflated swimbladder is to be expected as a general condition in leptocephali.

Leptocephalus larvae may compensate to some extent the lack of an air buoyancy organ by having a body consisting of about 90% water and also a high content of lipids. The lipids, due to their better oxygen

dissolving capacity may also serve to improve the oxygen supply of the tissues. But at the same time the willow-leaf shape may serve to improve the surface/volume ratio for cutaneous gas exchange compensating the lack of haemoglobin. During dramatic shrinking in a metamorphosis process blood becomes red, although according to Zwerger et al. (2002) it is not likely that the swimbladder might be inflated via the blood gas transport. As elvers (juvenile eels) are even larger than trout larvae, no size-dependent impediments of air gulping are to be expected. In addition the presence of surfactants may facilitate this process (Zwerger et al. 2002).

Leptocephalus larvae are known from Elopiform and Anguilliform fishes. In several galaxiids (Galaxiidae) there is a similar condition of translucency and lack of haemoglobin, even followed by a kind of metamorphosis in which the fish become smaller. Also the smelt (Osmeridae) presents a similar condition, albeit without this shrinking process. Translucency also might apply to some other Osmeriform fishes, although the blood condition has not yet been studied. The noodle fishes (Salangidae and Sundasalangidae) are extreme since, during the translucent condition, sexual maturity is reached (Roberts 1984).

Apart from the examples given, in several fishes with pelagic larvae (of small hatching size) the appearance of red blood as well as swimbladder inflation is considerably delayed, although not studied in detail. Their continuous need to struggle against sinking is in contrast to the less pelagic larvae like in Cyprinids which are red-blooded since before hatching and become buoyant early with the aid of a tiny swimbladder inflated by gas release from haemoglobin. Likely through the gas gland and the rete mirabile they can fill their swimbladder using the Root effect for secretion of oxygen into the swimbladder in a similar way as shown by Pelster and Randall (1998, see also Pelster 2001).

5.2. The “translucency Syndrome”

As discussed, translucent non red blooded fish larvae have a physiological deficiency in the mechanism of swimbladder inflation. They are impaired by the lack of haemoglobin (although we cannot exclude other less effective mechanisms of gas transport). Gulping air is hindered by the discussed problems of too high surface tension in relation to the small size. These traits appear as a whole functionally interconnected, accompanied by at least some of the compensatory mechanisms like struggle against sinking, need of much energy (Ueberschär 2006), heart hypertrophy (Busse and Campos 1996), etc. It resembles an assemblage of symptoms of a disease. Therefore we use the word from pathology: “syndrome” which means “symptoms running together”. Surely, it is a deficiency or a constraint, but it must also have an advantage.

A physiological deficiency can have an adaptive value. A well-known example is the sickle cell anaemia giving a certain resistance to malaria in man. For fish larvae the selection pressure against pigmentation (including red colour of haemoglobin shining through) might have been done by predators feeding on young fish.

5.3. Ecological implications

Many pelagic larvae contradictorily to what should be expected are worse in buoyancy than the ones dwelling amongst vegetation. The oil droplets in the yolk sac, as discussed above, are not sufficient for a substantial improvement of buoyancy. The alternative between either better buoyancy or being more cryptic for predators is clearly inclined to the last option. The result is the late swimbladder inflation and consequently a high energy demand for the struggle against sinking.

The larvae have to consume much food having additionally a digestive system of

low efficiency and finally have a high mortality by starvation. A compensation mechanism is the high fecundity to optimize the spreading out of larvae to extensive areas, so that at least a small fraction of them can exploit water bodies of high plankton densities, if by chance they have the opportunity to get into these areas. Experimental results show that many fish larvae have a typical diurnal rhythm in tryptic enzyme activity in correlation with their feeding (Ueberschär 1995; see also Ueberschär 2006). In the sardine there is a periodic antagonism between feeding and inflation of the swimbladder. Feeding occurs with a deflated gas bladder, while larvae in the inflated phase are handicapped in active swimming as well as in feeding, but in this condition they probably “rest” and save energy. The background for this periodicity is not clear (Hoss et al. 1989).

5.4. Delimitation of the translucency syndrome as a phylogenetic signal

The translucency syndrome, although having common traits depending on its specific physical and physiological constraints, it is a heterogeneous phenomenon. It must have evolved independently several times leading to similar appearances. Probably it is a diagnostic synapomorphic character only for smaller phylogenetic units. Further it can be quantitatively variable even within one species. In *Galaxias maculatus* the change from the white-bait stage to the pigmented juvenile can overcome at different body sizes. In the genus *Galaxias*, however, there is one species *G. zebratus*, with a developmental mode clearly corresponding to the red blooded early buoyant *Brachygalaxias* type. Morphological and molecular data given by McDowall and Waters (2004) fit with our result, but further research should be done before adopting nomenclatural decisions.

5.5. Flatulence and other mechanisms of inflation

Known mechanisms responsible for larval and/or adult swimbladder inflation are 1) gulping air, 2) gas transport via haemoglobin and liberation (as O₂) from the gas gland. In addition, CO₂ (or other dissolved gases) can be released by salting out in combination with lactic acid concentration, or produced by the pentose phosphate shunt in the gas gland cells. A further possibility for the use of gas in buoyancy is 3) flatulence, using bacterial gas production.

Brawn (1962) experimentally submitted (adult or subadult) herrings to increased pressure, and found that individuals fed with plankton, unlike those with empty stomachs, were able to recover buoyancy. Brawn (1962, 648) infers, that "...under certain conditions herring buoyancy may be restored by bacterial gas producing activity in the stomach". We observed that in *Galaxias maculatus* larvae gases sometimes appear in the rear part of the intestine, but in herring larval over-inflation problems mentioned above are due to ingestion of gas bubbles. It is generally accepted that adult herrings must surface to inflate their swimbladders because they lack a rete mirabile. This is in contradiction to the observation that they release excess gas through the anal duct when emerging from great depths. Furthermore, communication by means of their fast repetitive tic sounds (FRT) described by Wilson et al. (2004) implies gas consumption. There must be a source of gas. Wilson et al. (2004) went back to Brawn's (1962) hypothesis, but nature and source of gas is still not clarified. Pelster (2004) discusses further mechanisms for swimbladder gases coming from the breakdown of organic matter. Direct analyses of swimbladder gas exist only for some adult or subadult fish but not for larvae.

5.6. Temporary uselessness of the ductus pneumaticus

In physostomian fishes there are larvae of two size groups: relatively large types like Salmonids, or very small larvae. In the first, air gulping evidently is the way they initially fill their swimbladder, and is necessary for acquisition of buoyancy swimming. In the second group, we were not able to demonstrate this action (see constraints related with size and Fig. 8). Although they are physostomians, they have to inflate their swimbladder via the circulatory system, secreting oxygen by acidification and the Root (or Bohr) effect (see Pelster and Randall 1998). If they lack haemoglobin, they have to wait until they become red blooded or until they are large enough to swallow air. Apart of this, physostomian larvae initially inflating the swimbladder via the blood may acquire the ability to use the ductus pneumaticus to regulate buoyancy afterwards, as we could demonstrate in half grown zebrafish (*Danio rerio*) and *Pseudorasbora parva*.

5.7. Pre-adaptation to the physoclist condition

Summarizing, small physostomian larvae are temporarily functional physoclists. This condition possibly has been a key factor facilitating the degeneration of the ductus pneumaticus and giving raise to the evolution of the physoclist model of the majority of teleosts. In addition, fish groups that evolved a very numerous egg production had to do this at the expense of individual egg and larval size. Thus, the larvae had to pass through a surface tension constraint, which precludes direct swimbladder inflation. Haemoglobinless and translucent larvae are common in physostomians. Physoclists were primarily not considered in this study. When they are translucent red heart and liver are commonly observed, and when these are

camouflaged by silvery iridiophores, in most of them at least red vessels are visible.

Acknowledgements

We wish to thank Prof. Dr. Ralf Thiel, Universität Hamburg, Biozentrum Grindel und Zoologisches Museum, for the help in providing literature and material at the beginning of this study. Live and preserved smelt larvae were provided by Drs. Mathias Scholten, Arthur Bos, and Mara Petzenburg, at that time all at the Elbe-Labor, Hamburg-Altona, and Dr. Armin Kureck, Zoologisches Institut Universität Köln. We also wish to thank the colleagues from the Escuela de Acuicultura de Temuco, Chile: Drs. Juan Barile, Patricio Dantagnan, Alirio Borquez, Rolando Vega, Manuel Escudero for their help with infrastructure and hospitality and all help related with *Galaxias*. Especially the senior author thanks his relatives in Chile for their hospitality and all kind of help.

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