Differential processing of yolk proteins during oocyte hydration in marine fishes (Labridae) that spawn benthic and pelagic eggs

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ABSTRACT: The yolk proteins and free amino acids (FAA) were investigated during oocyte maturation in 4 species of Labridae with either benthic or pelagic eggs. Benthic eggs and oocytes of both types had a small FAA pool dominated by taurine, while pelagic eggs had a large and characteristic FAA pool with about equal amounts of indispensable and dispensable amino acids. Electrophoresis (SDS-PAGE) of the yolk proteins revealed the presence of high molecular weight yolk proteins in the oocytes of all 4 species. In 2 species with benthic eggs (Labrus bergylta, L. mixtus), no buildup of an FAA pool occurred (nmol µg–1 dry mass) during final maturation, and the yolk protein profile remained essentially unchanged. In a third species (Crenilabrus melops) with benthic eggs, partial degradation of a 112 kDa protein coincided with the buildup of a small FAA pool. In the species with pelagic eggs (Ctenolabrus rupestris), the buildup of a large FAA pool coincided with the disappearance of the 112 kDa yolk protein. The calculated yield of FAA by hydrolysis of the disappearing fraction of the 112 kDa yolk protein for both species matched well with the measured increase in the FAA pool. It is suggested that differential processing of the high molecular yolk proteins causes differences in teleost egg behaviour, benthic or pelagic.

KEY WORDS: Labridae · Wrasse · Oocyte hydration · Oocyte maturation · Proteolysis · Yolk proteins · Free amino acids

INTRODUCTION

It has recently been hypothesised that the success of the marine teleost group of fishes has a basis in biochemical changes occurring in the yolk compartment of the hydrating oocyte during maturation (Fyhn et al. 1999). It is argued that, due to the hyposmotic condition of the egg yolk of marine teleosts, there is an inescapable physiological demand for embryonic water to counteract the osmotic loss caused by the high salinity of the spawning environment. Thus, a reservoir of water must be transferred to the egg (i.e. the yolk) from the mother fish before spawning. The water problem is greatest during the early cellular phases of development, since marine teleost embryos apparently do not start drinking activity until shortly before or after hatching (Guggino 1980, Mangor-Jensen & Adoff 1987, Riis-Vestergaard 1987, Tytler et al. 1993).

The great majority of marine teleosts, regardless of systematic affinities, are oviparous and spawn predominantly pelagic eggs (Kendall et al. 1984). Other marine teleosts spawn benthic eggs. These fishes are termed pelagophils and benthophils, respectively (LaFleur & Thomas 1991, Finn et al. 2002). The Labridae are a family of exclusively marine teleosts that are highly diversified and include mostly tropical pelagophils but also some benthophils (Richards & Leis 1984, Nelson 1994). Seven species of Labridae have been recorded in Norwegian waters (Quinard & Pras
1986, Pethon 1989, Sayer & Treasurer 1996, Muus et al. 1999), and 4 are common in the coastal waters near Bergen. These are (taxonomy according to Wheeler 1992) the ballan wrasse *Labrus bergylta*, the cuckoo wrasse *L. mixtus*, the cokwing *Crenilabrus melops* and the goldsinny wrasse *Ctenolabrus rupestris*. The ballan and cuckoo wrasses are protogynous hermaphrodites and spawn benthic sticky eggs in male-guarded nests (Pethon 1989, Costello 1991). The cokwing also spawns benthic sticky eggs in male-guarded nests (Potts 1985, Skiftesvik et al. 1996, van der Meer & Leney 1998, Uglem & Rosenquist 2001), but it is considered a polygynous, batch-spawning gonochore (Dipper & Pullin 1979, Stone 1996, Uglem et al. 2000).

In contrast to these benthophils, the batch-spawning females of goldsinny wrasse are enticed to release pelagic eggs in a male-guarded territory (Hilldén 1984, Quignard & Pras 1986, Stone 1996). The goldsinny wrasse is also a polygynous gonochore (Hilldén 1984, Stone 1996). These 4 species were chosen as a related family group for the present study of the mechanism of oocyte hydration in marine teleosts.


The present contribution expands on this topic by showing a differential processing of the yolk proteins correlated with differences in the emerging pool of free amino acids in benthic and pelagic eggs of closely related marine fishes.

**MATERIALS AND METHODS**

**Sample collection.** Three benthophil species of Labridae (the ballan wrasse *Labrus bergylta*, the cokwing *Crenilabrus melops*) and 1 pelagophil species of Labridae (the goldsinny wrasse *Ctenolabrus rupestris*) were investigated. Females were collected by trapping and gill-netting during their spring and summer spawning seasons in coastal waters near Bergen, Norway (60°N, 5°E) and transported live to the Department of Zoology, University of Bergen, for dissection and analyses. Species were identified by colouring and meristic characters (Wheeler 1978, Quinard & Pras 1986, Pethon 1989, Muus et al. 1999). Only females that had both pre-hydrated oocytes (PH ooc) and ovulated eggs (OV eggs) simultaneously present in their ovaries were used for the mechanistic studies. A total of 5 *L. bergylta*, 2 *L. mixtus*, 4 *Crenilabrus melops*, and 4 *Ctenolabrus rupestris* were investigated. The killed fishes (blow to the head) were immediately measured, weighed, and dissected in a cold room (6°C). Standard lengths of the females were measured with a ruler, and their gonadosomatic index (GSI) was calculated according to Wootton (1990) following wet mass determination of the total ovaries and female bodies on a Sartorius (Type 1405 accuracy ± 0.1 mg) top balance. The GSIs of the studied females ranged from 7 to 11% except for 1 *L. bergylta* with a GSI of 15%.

Staging of the oocytes was established by size and degree of transparency, and they were classified as either PH ooc or OV eggs. Only the largest PH ooc were selected for dissection, and these were isolated and freed from adhering tissues with fine forceps. OV eggs were found free within the ovarian lumen, and were either rolled out or stripped from the excised ovaries. Individual PH ooc and OV eggs were freed of adhering fluid with lint-free filter paper (Kimwipes®), pooled in sealed Eppendorf tubes (N = 10 to 30 PH ooc and 10 to 40 OV eggs) for wet mass determination (Sartorius BP121S top balance, accuracy ±0.1 mg). Samples were then immediately frozen at –80°C, and were stored at –30°C until lyophilisation or extraction. Oocyte and egg dry masses were subsequently determined on a Cahn 25 Electrobalance (accuracy ± 1 µg) following 24 h of lyophilisation. Water contents (% wet mass and mg ind.⁻¹) were calculated from the difference between wet and dry mass. Oocyte and egg diameters were measured by placing separate sub-samples in FO medium (Wallace & Selman 1978) in order to avoid gravitational flattening and desiccation. Diameters were determined for 20 to 30 individual oocytes or eggs from each female with a calibrated Wild binocular microscope at 50 x magnification. No further analyses were performed on the sub-samples in FO media.

**Analytical procedures.** FAA analyses and quantitation and electrophoresis of proteins were carried out as follows:

**Free amino acid analyses:** Frozen samples were directly extracted (24 h, rotated at 4°C) in their original Eppendorf tubes with ice-cold 6 % trichloro-acetic acid
(TCA). The extracts were then centrifuged (14 000 \times g for 10 min, 4°C) and the supernatants were used for quantitation of FAA. FAA were determined by reversed-phase chromatography with a Gilson HPLC connected to an ASTED sample robot, fluorometric detection (OPA and FMOC reagents), Inertsil C3 column (thermostatted at 30°C), and every tenth sample was compared to external standards (mixture of 24 amino acids). Appropriately diluted TCA was used as blanks. The amount of FAA is expressed per individual (nmol ind.\(^{-1}\)) and the terminology of indispensable and dispensable amino acids is used (Harper 1983).

**Protein quantitation and electrophoresis:** The TCA precipitates were washed once in 6% TCA, then solubilised in 1.0 M NaOH under rotation for 24 to 48 h at room temperature. Equal volumes of double-distilled water (ddH\(_2\)O) were then added to give a final concentration of 0.5 M NaOH, and a further 24 h of solubilisation was allowed prior to protein determination. Protein content was measured without the addition of surfactant using Bio-Rad’s detergent compatible assay kit (Cat # 500-0112), which is a micro-modification of the Lowry technique (Lowry et al. 1951). Assays were conducted in quadruplicate using bovine serum albumin (BSA) as standard, and read at 750 nm in an Anthos Labtec HTII microplate absorption photometer.

For electrophoresis of yolk proteins, lyophilised samples were homogenised in ice-cold buffer (60 mM HEPES, 150 mM NaCl, 50 µg ml\(^{-1}\) aprotinin, pH 7.5), and centrifuged at 10 000 \times g, 5 min, 4°C; the supernatants were aspirated to clean Eppendorf tubes. Soluble protein concentration was estimated using the Bio-Rad detergent-compatible assay kit described above, and extracts were diluted to 0.8 µg µl\(^{-1}\) with reduced β-mercapto-ethanol, 0.0625 M Tris, 10% glycerol, 2% sodium dodecyl sulfate (SDS), 0.01% bromophenol blue, pH 6.8 and applied to duplicate 7.5% T, 3.3% C homogeneous acrylamide/bis-acrylamide gels (0.75 mm) using the buffer system of Schagger & von Jagow (1987). The stacking gel consisted of 4% T, 3.3% C acrylamide/bis-acrylamide. Samples were electrophoresed in a Bio-Rad Protean II cell at 95 V, 50 mA per gel for 80 min, and the protein bands were visualised with Coomassie Brilliant Blue G-250. For estimation of the apparent molecular weight of the visualised protein bands, Bio-Rad precision prestained markers at 250, 150, 100, 75, 50, 37, 25 15, and 10 kDa (Cat # 161-0372) were applied to both sides of the gel. The gels were scanned with an Epson GT-9600 scanner using transmitted light, and the relative mobility \(R_i\) of the standards and yolk proteins was measured digitally at 160× magnification. Estimates of the apparent \(M_i\) of yolk proteins were calculated from fifth-order polynomials fitted to plots of log\(M_i\) versus \(R_i\) \((r^2 = 0.9999)\). To improve the accuracy of the estimates of apparent \(M_i\) of the yolk proteins, the outermost lanes of the gel were excluded and the gels were run with excess anode buffer to reduce the smile effect. In addition, the 2 \(M_i\) standards on either side of the gels were checked for precise post-scan alignment. The relative fraction of each band in the gels was established by densitometry of the scanned gels using the National Institutes of Health (NIH) Image 1.62 Analysis software.

Statistical differences at the 5% level were determined via ANOVA according to the procedures of Sokal & Rohlf (2000).

**RESULTS**

The PH ooc of the species that spawn benthic eggs, i.e. *Labrus bergylta*, *L. mixtus* and *Crenilabrus melops*, were similar in size (620 to 670 µm), while those of *Ctenolabrus rupestris*, which spawns pelagic eggs, were significantly (\(p < 0.05\)) smaller (463 ± 38 µm: Table 1). The relative water content of the PH ooc was 61 to 62% of wet mass in both benthophils and pelagophils, although the value for *L. bergylta* was higher. The most noteworthy change that occurred during oocyte maturation was the greater swelling of the pelagic OV eggs in *C. rupestris*, with wet mass increasing by a factor of 8.4, compared to a factor of 2 to 3 for the benthic eggs. This was due to a significantly (\(p < 0.05\)) greater hydration of the pelagic OV eggs, which reached a relative water content of ~93% compared to 76 to 78% for the benthic OV eggs. The dry mass increased during oocyte maturation in all fishes, but much less than the concomitant increase in wet mass.

The protein content of the PH ooc of the 2 benthophils *Labrus bergylta* and *L. mixtus* increased significantly (\(p < 0.05\)) during the hydration period, while that of the third benthophil, *Crenilabrus melops*, also increased, although not significantly. In contrast, the protein content of the PH ooc of the pelagophil *Ctenolabrus rupestris* significantly (\(p < 0.05\)) decreased in the OV eggs (Fig. 1). Although, due to their smaller size, the total protein content of the PH ooc of *C. rupestris* was lower than that of the benthophils’, the mass-specific protein fraction was similar (68% of dry mass compared to 56–70% of dry mass for the 3 benthophils). During the hydration period, the mass-specific protein content did not change significantly (\(p > 0.05\)) in the OV eggs of the 3 benthophils, but declined significantly (\(p < 0.05\)) in the OV eggs of *C. rupestris*.

The FAA contents of both benthophil and pelagophil PH ooc were low and characterised by a dominance of the non-proteinic amino acid taurine, which made up 30 to 45% of the total FAA pool (Fig. 2). A small FAA
pool with taurine dominance was also found in the benthic OV eggs, although in the case of Crenilabrus melops the pool increased (Fig. 3). The most remarkable change, however, was in the pelagic OV eggs of Ctenolabrus rupestris, in which the FAA content increased strongly (factor of 8.6; Table 2) and thus also changed their profile compared to the PH ooc profile (Fig. 2). When normalised with respect to dry mass, the FAA content of C. rupestris increased significantly (p < 0.05) from ~4% of dry mass in the PH ooc to 19% in the OV eggs (Fig. 3). In comparison, no significant changes occurred in the FAA content of the 2 Labrus species during the hydration period, while in Ctenolabrus melops it increased significantly (p < 0.05) from 3 to 5% of the dry mass (Fig. 3). The appearance of the large FAA pool in the pelagic OV eggs of C. rupestris was due to increases in virtually all of the measured FAA, but particularly of the indispensable amino acids such that the contents of indispensable and dispensable amino acids became almost equal (Fig. 2, Table 2).

In comparison, the distribution between indispensable and dispensable amino acids for the benthophils remained highly skewed, with a dominance of dispensable amino acids due to the high levels of taurine.

Table 1. Labridae. Biometric and gravimetric changes during oocyte hydration of 4 species. PH ooc: pre-hydrated oocyte; OV egg: ovulated egg. N: number of replicate measurements of pooled samples; numbers in parentheses: total number of dissected oocytes or eggs in the pooled samples; SL: standard length. Swell factors calculated by dividing the measured wet mass of the ovulated eggs by that of the pre-hydrated oocytes

<table>
<thead>
<tr>
<th>Variable</th>
<th>PH ooc</th>
<th>OV egg</th>
<th>Swell factor</th>
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<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean ± SD</td>
<td>N</td>
</tr>
<tr>
<td>Labrus bergylta (benthic)</td>
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<tr>
<td>2 females, SL: 25 + 29 cm</td>
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<tr>
<td>Diameter (µm)</td>
<td>60</td>
<td>670 ± 50</td>
<td>60</td>
</tr>
<tr>
<td>Wet mass (µg ind.⁻¹)</td>
<td>16 (200)</td>
<td>139 ± 25</td>
<td>16 (225)</td>
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<tr>
<td>Dry mass (µg ind.⁻¹)</td>
<td>8 (99)</td>
<td>41 ± 6</td>
<td>8 (109)</td>
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<tr>
<td>H₂O (%)</td>
<td></td>
<td>70.5 ± 2.4</td>
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<td>Labrus mixtus (benthic)</td>
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<td>1 female, SL: 16 cm</td>
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<tr>
<td>Diameter (µm)</td>
<td>30</td>
<td>620 ± 30</td>
<td>30</td>
</tr>
<tr>
<td>Wet mass (µg ind.⁻¹)</td>
<td>8 (144)</td>
<td>114 ± 7</td>
<td>8 (153)</td>
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<tr>
<td>Dry mass (µg ind.⁻¹)</td>
<td>4 (63)</td>
<td>44 ± 3</td>
<td>4 (91)</td>
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<tr>
<td>H₂O (%)</td>
<td></td>
<td>61.4 ± 1.6</td>
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<tr>
<td>Crenilabrus melops (benthic)</td>
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<td>2 females, SL: 17 + 18 cm</td>
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<tr>
<td>Diameter (µm)</td>
<td>46</td>
<td>630 ± 60</td>
<td>32</td>
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<tr>
<td>Wet mass (µg ind.⁻¹)</td>
<td>16 (286)</td>
<td>110 ± 26</td>
<td>16 (162)</td>
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<tr>
<td>Dry mass (µg ind.⁻¹)</td>
<td>7 (146)</td>
<td>43 ± 18</td>
<td>7 (83)</td>
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<tr>
<td>H₂O (%)</td>
<td></td>
<td>61.0 ± 5.8</td>
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<tr>
<td>Ctenolabrus rupestris (pelagic)</td>
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<tr>
<td>2 females, SL: 8.1 + 10.5 cm</td>
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<tr>
<td>Diameter (µm)</td>
<td>60</td>
<td>463 ± 38</td>
<td>60</td>
</tr>
<tr>
<td>Wet mass (µg ind.⁻¹)</td>
<td>16 (274)</td>
<td>37 ± 9</td>
<td>16 (189)</td>
</tr>
<tr>
<td>Dry mass (µg ind.⁻¹)</td>
<td>6 (106)</td>
<td>14 ± 2</td>
<td>8 (101)</td>
</tr>
<tr>
<td>H₂O (%)</td>
<td></td>
<td>62.2 ± 4.3</td>
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Fig. 1. Labridae. Protein content (mean ± SD) of pre-hydrating oocytes [PH ooc] and ovulated eggs (OV egg) of 4 species spawning benthic or pelagic eggs.
Fig. 2. Labridae. Content (means ± SD) of free amino acids in pre-hydrating oocytes (PH ooc) and ovulated eggs (OV egg) of 4 species spawning benthic or pelagic eggs. Values have been normalised for size differences by multiplying the molar values of each amino acid with its molecular mass and expressing as a weight percent of the total oocyte or egg dry mass.
Electrophoresis of the yolk proteins revealed a coincidence between a band shift and the appearance of the FAA pool during oocyte hydration. For *Labrus bergylta*, which maintained constant mass-specific amounts of FAA (Fig. 3), the major protein bands at 107, 100, and 73 kDa, and a minor band at 34 kDa in the PH ooc were also present in the OV eggs (Fig. 4). Similarly, for *L. mixtus*, which also showed no change in the mass-specific FAA content, the major bands at 112, 91, 71 kDa and minor bands at 22 and 20 kDa were also found in the OV eggs, although the 112 kDa protein may have decreased in quantity. In contrast, significant changes occurred in the protein profiles of *Crenilabrus melops*, in which in particular a major band at 112 kDa (41% of the proteins are localised at this band, as determined by densitometry) disappeared, while bands at 72 and 22 kDa appeared.

The most notable changes in the electrophoretic yolk protein profile occurred in the pelagic eggs of *Ctenolabrus rupestris* concurrent with the large increase in the FAA pool. The major band at 112 kDa (43% of the proteins are localised at this band, as determined by densitometry) and the less dominant bands at 97, 65, and 57 kDa, as well as the minor bands at 77, 22, and 20 kDa in the PH ooc, all disappeared during the hydration period. In contrast, new bands (in particular a major band at 21 kDa but also minor bands at 92 and 75 kDa) were found in the OV eggs.

**DISCUSSION**

Group-synchronous ovaries and batch-spawning are typical for oviparous marine pelagophils, i.e. the majority of marine teleosts (Kendall et al. 1984), but batch-spawning also occurs in freshwater benthophils such as the crucian carp *Carassius carassius* (Aho & Holopainen 2000) or the freshwater goby *Micropercops swinhonis* (Iwata et al. 2001). In batch-spawning, several ‘batches’ of the oocytes are induced to undergo final maturation at different times, with the result that several egg batches are released within a reproductive season. The simultaneous presence of both OV eggs and PH ooc (the latter being in the majority) in each of the 4 species of Labridae studied reveals that these fishes have group-synchronous ovaries. Both the benthophil *Crenilabrus melops* and the pelagophil *Ctenolabrus rupestris* are recognised batch-spawners (Dipper & Pullin 1979, Dipper 1984, Stone 1996, Uglem et al. 2000), but to our knowledge little information exists on the spawning habits or mating system of the 2 benthophils *Labrus bergylta* and *L. mixtus*, although their group-synchronous ovaries suggest that they have batch-spawning potential. Further studies will clarify their spawning habits and mating system.

The physiological data show that the studied species of Labridae utilise different mechanisms for hydrating their oocytes, depending on
whether benthic or pelagic eggs will be produced. In the 2 benthophil Labrus species, virtually no change occurred in the electrophoretic protein profiles or in the FAA pool during oocyte maturation. Rather, these fishes (L. bergylta and L. mixtus) continued sequestering yolk proteins during final maturation, and the concomitant hydration was relatively small. Although we cannot preclude that the PH ooc of L. bergylta were in a mid-vitellogenic phase, a continued protein sequestration is known to occur during the early phases of oocyte maturation (germinal vesicle migration) in other benthophil (Wallace & Selman 1985, Thorsen et al. 1993, Tyler & Sumpter 1996), and the degree of hydration is similar to earlier reports on marine benthophils (Craik & Harvey 1986, Greeley et al. 1986). Furthermore, the early phases of oocyte maturation in some teleosts may last several weeks (Mylonas et al. 1997).

Intriguingly, Crenilabrus melops, which also spawns benthic eggs, showed an altered yolk protein profile and some increase in the FAA pool during oocyte hydration. This indicates participation of another hydration mechanism. The increase in the FAA pool coincided with the disappearance of the 112 kDa protein band in the PH ooc and the appearance of a band at 72 kDa and another band at 22 kDa in the OV eggs. Recently, Matsubara et al. (1999), studying the pelagophile Verasper moseri, demonstrated that 2 high molecular weight yolk proteins (107 and 94 kDa, respectively) are the heavy chains of lipovitellins derived from 2 forms of vitellogenin. Similar studies for the pelagophile Melanogrammus aeglefinus argued that the observed high molecular weight yolk proteins were also derived from 2 forms of vitellogenin (Reith et al. 2001). In both these species (Matsubara et al. 1999, Reith et al. 2001) only one of the high molecular weight proteins was hydrolysed, while the other yolk protein was nicked. Based on the present methodology, we cannot determine whether the observed yolk proteins of C. melops are homologous to those of V. moseri or M. aeglefinus, although the change in the electrophoretic profile with the concurrent rise in FAA during oocyte maturation of C. melops suggests a similar mechanism in the species. This notion is strengthened by the experiments of Thorsen & Fyhn (1996), who demonstrated with oocyte cultures of the plaice Pleuronectes platessa, a teleost with pelagic eggs, that the increase in egg FAA pool was not caused by transport from an extracellular source. Furthermore, since densitometry of C. melops PH ooc proteins revealed that the 112 kDa band that disappeared during oocyte hydration accounted for 41% of the 30.4 µg ind.\(^{-1}\) yolk protein, hydrolysis of an 18 kDa fragment would be necessary to generate an 18 nmol FAA ind.\(^{-1}\) increase in the OV eggs, whereas actually an increase of 20 nmol FAA ind.\(^{-1}\) was found during this period (Table 2). This calculation is based on an average polymerised amino acid molecular weight of 110 g mol\(^{-1}\) for the amino acid pool in question, and taurine is excluded. The loss of such an 18 kDa from the original 112 kDa protein could explain the novel OV egg proteins of 72 and 22 kDa (Fig. 4). Further molecular characterisation is necessary to verify this possibility.

For the pelagophile Ctenolabrus rupestris, in which a larger increase in the FAA pool occurred, there seems to be a greater contribution of the 112 kDa yolk protein. A major portion of this protein (43% of the 9.5 µg oocyte proteins) seems to be split or fully hydrolysed, since mainly a protein band of ~21 kDa exists in the OV eggs. Using the same calculation procedure as for Crenilabrus melops, the hydrolysis of the disappearing 91 kDa fragment should generate 30 nmol FAA ind.\(^{-1}\), whereas actually 35 nmol ind.\(^{-1}\) was measured (Table 2). The fact that there is a substantial
increase in the indispensable amino acids in the egg FAA pool supports the inference of yolk protein hydrolysis. At present, however, we are unable to account for the disappearance of the 64 and 57 kDa bands during the hydration phase. Furthermore, the calculations do not take into account sequence-specific binding of Coomassie Brilliant Blue G-250, or of the presence of highly anionic proteins such as the phosvitins which may be present in teleost yolk (Wallace & Bergovac 1985, Murakami et al. 1991, Losso et al. 1993, Goulas et al. 1996, Matsubara et al. 1999, 2000, Wang et al. 2000).

It has been well documented that vitellogenin is the major source of yolk proteins in ostecichthyian fishes (Ng & Idler 1983, Mommsen & Walsh 1988, Specker & Sullivan 1994, Johanning & Specker 1995, Tyler & Sumpter 1996), but only recently have multiple forms of vitellogenin been reported in teleosts. Multiple forms of vitellogenin, have been found in freshwater benthophils (Kishida & Specker 1993, Lee et al. 1994, Trichet et al. 2000, Wang et al. 2000), in marine benthophils (LaFleur et al. 1995), as well as in marine pelagophils (Matsubara et al. 1999, Reith et al. 2001). These latter studies have further mapped some of the oocyte proteins to the different vitellogenin cDNAs and argued that their hydrolysis was part of the mechanism that generates the FAA pool in pelagic fish eggs. It seems probable, based on the findings in the present study, that the Labridae also possess at least 2 forms of vitellogenin giving rise to the 2 high molecular weight proteins (107 to 112 kDa and 90 to 96 kDa bands) found in the PH ooec of all 4 species.

Since the Labridae species are found exclusively in marine habitats, with the majority spawning pelagic eggs in tropical environments (Richards & Leis 1984), the 3 benthophil species in the present study are apparently among the minority in this family of fishes. Demersal eggs of marine fishes have been associated with cooler coastal environments, but are also phylogenetically an old character associated with ancient groups such as osmerids and clupeoids (Hempel 1984). As members of the order Perciformes, the Labridae are of recent evolutionary origin (Long 1995, Helfman et al. 1999), and thus comply with the suggestion that demersal spawning has developed independently in several groups (Hempel 1984). The differential processing of the yolk proteins concurrent with the appearance of a variable FAA pool, as observed in the present study, may have intriguing implications for the evolution of the benthic or pelagic character of teleost eggs.

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