Using sagittal otoliths and eye diameter for ecological characterization of deep-sea fish: *Aphanopus carbo* and *A. intermedius* from NE Atlantic waters

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SUMMARY: The sagittal otolith and eye diameter of two sympatric species of the genus *Aphanopus*, *A. carbo* and *A. intermedius*, from North Atlantic waters were investigated. The study objectives were to find morphometric variables of otoliths to identify the two species and relate ecomorphological characters of otolith and eye diameter to the depth distribution of each species. The otoliths of the two species are very similar, although significant differences in shape, otolith height and weight and the *sulcus acusticus* area were detected. The ratio between eye diameter and cephalic length (*ED:CL* ratio) was also significantly different. In both analyses *A. carbo* obtained higher values, which suggests that *A. carbo* and *A. intermedius* live in different spatial niches, and that *A. carbo* inhabits deeper waters. The ratio between the *sulcus* and otolith areas (*S:O*) was low compared to other fish species, which suggests that the hearing capacity of *Aphanopus* spp. is adapted to low frequency sound. This adaptation may be related to the oceanographic conditions in which these species live, and in particular to the characteristics of sound transmission in the “SOFAR channel”.

Keywords: ecology, morphometry, otolith, *Aphanopus carbo*, *Aphanopus intermedius*, teleosts.

RESUMEN: Uso del otolito sagitta y el diámetro del ojo para la caracterización ecológica de peces de profundidad: *Aphanopus carbo* y *A. intermedius* del Atlántico nororiental. – El otolito sagitta y el diámetro del ojo de dos especies simpáticas del género *Aphanopus*, *A. carbo* y *A. intermedius*, del Atlántico Norte fueron analizados. Los objetivos del estudio se centraron en encontrar las variables morfométricas del otolito que pudiesen ser empleadas en la diferenciación de ambas especies y en establecer una correspondencia entre la profundidad de distribución de las especies y las características ecomorfológicas del otolito y del diámetro del ojo. Los otolitos de ambas especies resultaron ser parecidos, si bien se detectaron diferencias significativas en la forma, en el peso y la anchura del otolito y en el área del *sulcus acusticus*. La proporción entre el diámetro del ojo y la longitud cefálica (*ED:CL*) también puso de manifiesto diferencias significativas entre ambas especies. En ambos análisis, los resultados indicaron que *A. carbo* alcanza valores mayores. Ello proporciona evidencias para poder afirmar que *A. carbo* y *A. intermedius* viven en nichos espaciales diferentes, habitando *A. carbo* aguas más profundas. La proporción entre el área del *sulcus* y el área del otolito (*S:O*) alcanzó valores bajos en comparación con otras especies, lo cual sugiere que la capacidad auditiva de *Aphanopus* spp. está adaptada a sonidos de baja frecuencia. Dicha adaptación podría estar relacionada con las condiciones oceanográficas donde viven estas especies y, en particular, con las características de la transmisión del sonido dentro del denominado “canal SOFAR”.

INTRODUCTION

The black-scabbard fish Aphanopus carbo Lowe, 1843, has been commercially fished in the waters of the archipelago of Madeira for more than 150 years. This makes it the oldest deep-sea commercial fishery in the world (Shotton, 2005). Nowadays, it is also fished in continental fishing grounds off Sesimbra waters, Portugal (Bordalo-Machado et al., 2009). This species also appears as by-catch in the trawl fishery in the west of the British Isles, along the Middle-Atlantic Ridge and at Corner Rise (Gordon, 2001). Its annual catches reach up to 14000 tonnes, reported mainly by the Portuguese and French fleets (Martins et al., 1994; Martins and Ferreira, 1995; Morales-Nin et al., 2002). Due to its commercial importance, many studies on fishery ecology have been carried out (Howe et al., 1979; Clarke et al., 2003; Gordo, 2009). These studies suggest that this species undergoes horizontal migration movements associated with its inner ontogenic cycle. In addition, juveniles probably move from the northwest of Scotland to the Portuguese continental coast in search for food during their first years of life. When they reach sexual maturity, they migrate to the Macaronesian archipelagos, the only known reproductive areas (Martins et al., 1994; Machado et al., 1998; Figueiredo et al., 2003; Pajuelo et al., 2008; Neves et al., 2009). This means that the North Atlantic population may be considered as a population unit, independently of management and assessment aspects for regulating its fisheries.

At the beginning of the 1980s a series of descriptions of new species of Aphanopus started to appear; one of them, A. intermedius Parin, 1983, is partially sympatric with A. carbo (Nakamura and Parin, 1993; Parin, 1995). Recent morphologic and genetic studies have demonstrated that the two species are present in the Azores, Madeira, the Canary Islands and off Morocco and the western Sahara (M. Biscoito, com. pers.). The external identification of these species is currently impossible, since it is necessary to count dorsal fins and vertebrae in order to distinguish them (Nakamura and Parin, 1993; Parin, 1995). The bathymetric distribution of A. carbo ranges between 200 and 2300 m, although the depth of the most frequent distribution is around 1000 m; whereas A. intermedius lives from 300 to 1500 m (Nakamura and Parin, 1993; Lorance et al., 2001; Morales-Nin and Sena-Carvalho, 1996; Pajuelo et al., 2008). However, due to the current confusion between the two species, it is not possible to confirm precisely the depth distribution of each species.

According to ecomorphological premises, some characters should have a close relationship with the environmental factors that form the habitat of the organism (Bock, 1990; Norton et al., 1995). Therefore, the study of morphological features related to the sensorial perception of environmental stimuli (Blaxter, 1988; Weissburg, 2005) may help us to understand which adaptive features can indicate a certain ecological segregation (Schoener, 1974; Lombarte and Aguirre, 1997; Seehausen et al., 2008).

The sensorial characters with ecological implications most studied are: i) the eye size and the eye’s inner structures (Fernald, 1988; Bozzano et al., 2001), and ii) the sagittal otoliths, the main structure of the inner ear in Teleostean non-ostariophysian fish (Aguirre and Lombarte, 1999; Volpedo and Echevaría, 2003; Tuset et al., 2003, Lombarte and Cruz, 2007). Lombarte (1992) indicated differences in relative size of the sulcus acusticus between Merluccius capensis and M. paradoxus, and concluded that the species that lives at greater depths, M. paradoxus, obtained higher values. Schulz-Mirbach et al. (2008) also found differences in otolith morphology between surface and cave populations of Poecilia mexicana. Consequently, the goal of this paper is to hypothesize on the spatial niche of the two species by analyzing (i) the otolith morphometry, (ii) the ratio between the sulcus acusticus area and otolith area, and (ii) the ratio between the eye diameter and cephalic length, in order to determine whether these characters can indicate the ecological segregation of the two species.

MATERIALS AND METHODS

Sampling and data collection

A total of 137 specimens of Aphanopus carbo and A. intermedius were collected off Sesimbra (mainland Portugal), the Azores, Madeira, the Canary Islands and off the coasts of Morocco and the western Sahara (Fig. 1). The individuals from Sesimbra, the Azores and Madeira were taken randomly from the commercial fishery. In the other locations, specimens were
obtained from experimental fishing. As it is difficult to distinguish the species using external morphologic characters, all specimens were genetically identified according to the method developed by Stefanni and Knutsen (2007).

Cephalic length (CL in mm) and eye diameter (ED in mm) were measured (Fig. 2) for all individuals (the total, standard and furcal lengths were not considered for this study because in many specimens the posterior end was damaged), and otoliths were removed, washed, dried and stored in plastic vials. The otoliths of the two species were described morphologically according to the terminology proposed by Tuset et al. (2008).

All otoliths were weighed (OW) to the nearest 0.01 mg. Otoliths from the right side of the fish were orientated with the sulcus side up. Their images were digitized using a microscope attached to an image analyzer with a magnification of 1.5-2.5x depending on the size of the otolith. Otolith area (OA in mm²), length (OL in mm), height (OH in mm) and perimeter (OP in mm) and sulcus area (SA in mm²) (Fig. 2) were measured using Image-Pro Plus version 4.1.0 software (Media Cybernetics, Inc.). To avoid fish size effecting the analyses, the individuals were truncated from 173 mm CL.

**Data analysis**

To detect the specific ontogenetic changes in otolith morphometry and visual perception, all variables were fitted by a power function Y = aX^b. Statistical differences in the regression slopes between species were tested by means of an ANCOVA, treating species as the main factor and length (or otolith height) as the covariate. One-way (ANOVA) was used to compare the eye diameter:cephalic length (ED:CL) and sulcus area:otolith area (S:O) ratios among size groups (CL in mm, <200, 201-240, 241>) and a post-hoc Tukey’s test was applied; whereas a Student’s t-test was used for comparisons according to species and size class (Zar, 1996).

**RESULTS**

The otolith morphology of the two species is very similar. According to Tuset et al. (2008): the ventral margin is sinuate to crenate. Sulcus acusticus: heter-
osulcoid, ostial, median. Ostium: funnel-like, shorter than the cauda. Cauda: tubular, straight, ending half way to the posterior margin. Anterior region: peaked; rostrum short, broad, pointed; antrostrum absent or short, narrow, pointed; excisura wide with or without a deep and acute notch. Posterior region: round. Nevertheless, it seems clear that the otolith shape is different for the two species, as *A. carbo* is more oblong and less elliptic than *A. intermedius* (Fig. 3).

The descriptive statistics of all variables are summarized in Table 1. Although the mean cephalic length was higher in *A. intermedius*, the mean of the rest of variables was greater in *A. carbo* (ANOVA, F= 1.735, P< 0.05). However, Student’s t-test showed significant differences (t= 3.844, P<0.001) between species, and *A. carbo* obtained higher values (mean and standard deviation, 0.221 ± 0.022) than *A. intermedius* (0.203 ± 0.026) (Fig. 4).

Otolith length was not a good predictor of otolith weight (r²<0.60), while the variation in otolith height values was greater than 77% for both species (Table 2, Fig. 5). The ANCOVA test showed significant differences between species for both relationships (OH-OW, F= 3.350; OL-OW, F= 19.849; P<0.05). However, all otolith variables showed significant correlations with cephalic length. The variable most strongly related to fish size was the otolith weight, with 84.9% of the variability in *A. intermedius* and 69.5% in *A. carbo* explained by the model. The comparison with the slopes indicated significant differences between species for the CL-OH (ANCOVA, F= 5.663, P<0.05), CL-OW (F= 5.106, P<0.05) and CL-SA (F= 9.368, P<0.05) relationships, although slopes of all equations were higher for *A. carbo* (Table 2, Fig. 6). In this sense, more noticeable differences were detected in the relative increase in weight and the sulcus acusticus area, as the distance between slopes increased as the cephalic length became larger.

The S:O ratio did not vary significantly among size classes in *A. carbo* (ANOVA, F= 2.914, P> 0.05), although a positive tendency was observed with the increase in fish size. However, significant differences were detected in *A. intermedius* (ANOVA, F= 3.334, P= 0.046). The Tukey’s test grouped the size classes 180-220 and 220-260 (Fig. 7). Finally, the ratio analysis did not show significant differences between species for each size class (Student’s t-test, t= 0.609 in 180 mm, t= 0.493 in 220 mm, t= 1.956 in 260 mm, P>0.05) (Fig. 7).

**Table 1.** – Descriptive statistics of fish and otolith variables in Aphanopus spp. CL, cephalic length; ED, eye diameter; OA, otolith area; OH, otolith height; OL, otolith length; OP, otolith perimeter; OW, otolith weight; SA, sulcus area.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Aphanopus carbo</th>
<th></th>
<th></th>
<th></th>
<th>Aphanopus intermedius</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Min.</td>
<td>Max.</td>
<td>Mean</td>
<td>sd</td>
<td>n</td>
<td>Min.</td>
<td>Max.</td>
</tr>
<tr>
<td>CL</td>
<td>71</td>
<td>174</td>
<td>250</td>
<td>210.89</td>
<td>17.65</td>
<td>42</td>
<td>173</td>
<td>270</td>
</tr>
<tr>
<td>ED</td>
<td>71</td>
<td>33</td>
<td>59</td>
<td>46.5</td>
<td>5.74</td>
<td>42</td>
<td>30</td>
<td>62</td>
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<td>OA</td>
<td>71</td>
<td>19.01</td>
<td>35.16</td>
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<td>15.99</td>
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<td>OH</td>
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<td>3.26</td>
<td>4.69</td>
<td>3.97</td>
<td>0.36</td>
<td>42</td>
<td>2.97</td>
<td>4.64</td>
</tr>
<tr>
<td>OL</td>
<td>71</td>
<td>7.35</td>
<td>10.59</td>
<td>8.69</td>
<td>0.6</td>
<td>42</td>
<td>6.82</td>
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<td>OP</td>
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<td>24.97</td>
<td>21.49</td>
<td>1.6</td>
<td>42</td>
<td>16.54</td>
<td>24.56</td>
</tr>
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<td>OW</td>
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<td>22.4</td>
<td>72.00</td>
<td>42.22</td>
<td>13.09</td>
<td>42</td>
<td>17.6</td>
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<tr>
<td>SA</td>
<td>71</td>
<td>0.99</td>
<td>5.03</td>
<td>2.32</td>
<td>0.94</td>
<td>42</td>
<td>0.73</td>
<td>3.79</td>
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</table>

Fig. 4. – Box plots (maximum, minimum, upper and lower quartiles) for the eye diameter:cephalic length ratio (ED:CL) in Aphanopus spp.

Fig. 5. – Power relationships between otolith weight and otolith length and height in *A. carbo* (black dots) and *A. intermedius* (white squares).
DISCUSSION

The present study provides evidence to assert that *A. carbo* and *A. intermedius* are adapted to living in different spatial niches, which confirms the previous assumption that *A. carbo* inhabits deeper waters, although overlapping occurs (Nakamura and Parin, 1993; Morales-Nin and Sena-Carvalho, 1996).

Fish have a variety of different sensory receptors that enable them to glean a good deal of information about their sensory environment (Atema et al., 1988). The vision of deep-sea animals inhabiting different

Table 2. – Regression parameters of the relationships by species and ANCOVA test comparing the slopes between species. CL, cephalic length; OA, otolith area; OH, otolith height; OL, otolith length; OP, otolith perimeter; OW, otolith weight; SA, sulcus area.

<table>
<thead>
<tr>
<th>Relationship</th>
<th>Aphanopus carbo</th>
<th>Aphanopus intermedius</th>
<th>ANCOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>CL-OA</td>
<td>71</td>
<td>0.019</td>
<td>1.341</td>
</tr>
<tr>
<td>CL-OH</td>
<td>71</td>
<td>0.035</td>
<td>0.886</td>
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<tr>
<td>CL-OL</td>
<td>71</td>
<td>0.508</td>
<td>0.530</td>
</tr>
<tr>
<td>CL-OP</td>
<td>71</td>
<td>0.469</td>
<td>0.715</td>
</tr>
<tr>
<td>CL-OW</td>
<td>71</td>
<td>3.4*10^6</td>
<td>3.048</td>
</tr>
<tr>
<td>CL-SA</td>
<td>71</td>
<td>6.3*10^6</td>
<td>3.243</td>
</tr>
<tr>
<td>CL-OP</td>
<td>71</td>
<td>0.048</td>
<td>3.121</td>
</tr>
<tr>
<td>OH-OW</td>
<td>71</td>
<td>0.723</td>
<td>2.923</td>
</tr>
</tbody>
</table>

Fig. 6. – Power relationships between cephalic length and otolith variables in *A. carbo* (black dots) and *A. intermedius* (white squares).
depths are matched to a gradual change in visual scene from an extended source to point source. Species living in the mesopelagic zone (150-1000 m), which is dimly lit by down-welling daylight, have comparatively large eyes relative to head size (Warrant and Locket, 2004). It is known that *A. carbo* — or *A. intermedius* due to the current confusion in the North Atlantic — migrates vertically at night to the upper water layer to feed on crustaceans, cephalopods and benthopelagic, mesopelagic and pelagic fish depending on its ontogenic cycle (Mauchline and Gordon, 1984; Pshenichny et al., 1986; Figueiredo et al., 2003). This circadian migration to layers with more light should affect the vision pattern as this is influenced by daylight. In fact, the morphology and size of the eyes in *Aphanopus* spp. is similar to other trichiurids from shallower waters. Although they live below 1000 m, the following premise could apply to them: larger eyes (relative to body size) are related to a deeper depth distribution. Our results show that the eyes of *A. carbo* are larger, and therefore it can be hypothesized that either it inhabits deeper waters or it does not reach surface waters in vertical migration, or both. The reason why *Aphanopus* spp. occurs in deeper waters in the Canary Islands and Madeira may be in order to avoid overlapping with the trophic niches of other trichiurids from shallower waters. Although they live below 1000 m, the following premise could apply to them: larger eyes (relative to body size) are related to a deeper depth distribution. Our results show that the eyes of *A. carbo* are larger, and therefore it can be hypothesized that either it inhabits deeper waters or it does not reach surface waters in vertical migration, or both. The reason why *Aphanopus* spp. occurs in deeper waters in the Canary Islands and Madeira may be in order to avoid overlapping with the trophic niches of other trichiurids from shallower waters. Although they live below 1000 m, the following premise could apply to them: larger eyes (relative to body size) are related to a deeper depth distribution. Our results show that the eyes of *A. carbo* are larger, and therefore it can be hypothesized that either it inhabits deeper waters or it does not reach surface waters in vertical migration, or both. The reason why *Aphanopus* spp. occurs in deeper waters in the Canary Islands and Madeira may be in order to avoid overlapping with the trophic niches of other trichiurids from shallower waters.
is deeper in the subtropics and close to the surface in high latitudes (Rogers and Cox, 1988). It seems logical to consider that the development of the *sulcus acusticus* is an adaptation to habitat, since different prey will produce signals that can be detected from closer or farther away. There are aspects of the otolith structure, such as its thickness, the depth of the *sulcus* and the shape of the otolith itself, that do not have a known impact on the hearing capabilities (Popper and Lu, 2000; Reichenbacher *et al.*, 2007). Future research should be focused on developing three-dimensional techniques to help us better understand the relation between fish ecology and otolith morphology and hearing capabilities.

**ACKNOWLEDGEMENTS**

Many people helped us in the data collection and sampling involved in this study. We thank José Ignacio Santana, José Antonio Pérez-Péñalvo, Olga Ayza, and Antonio García-Mederos from the Instituto Canario de Ciencias Marinas (Gran Canaria, Spain); Manuel Biscucho from the Museu Municipal do Funchal (História Ciencias Marinas (Gran Canaria, Spain); Manuel Bischof, H. Aguirre, H. and A. Lombarte. – 1999. Ecomorphological comparisons of sagittae in *Mullus barbatus* and *M. surmuletus*. *J. Fish Biol.*, 55: 105-114.


Received March 9, 2009. Accepted April 15, 2010. Published online September 23, 2010.