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Habitat specific growth rates and condition indices for the sympatric soles *Solea solea* (Linnaeus, 1758) and *Solea senegalensis* Kaup 1858, in the Tagus estuary, Portugal, based on otolith daily increments and RNA-DNA ratio

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Summary

Habitat specific growth rates and condition indices were estimated for *Solea solea* and *Solea senegalensis*, in two nursery areas within the Tagus estuary, at the end of the estuarine colonization process, in 2005. While in the uppermost nursery area the two species of sole live in sympatry, in the lower nursery only *S. senegalensis* is present. Daily increments of left lapillar otoliths were used to estimate age (in days) and determine growth rates (mm per day). Condition indices were assessed through RNA-DNA ratio in muscle samples. Growth rates were higher for *S. senegalensis* (0.970and 1.180 mm per day in nursery A and B, respectively) than for *S. solea* (0.767 mm per day in nursery A). Growth rates of *S. senegalensis* from the uppermost nursery area were lower when compared to those obtained for the other nursery. The RNA / DNA condition index followed the general trend given by the growth rate estimates, i.e. values were higher for *S. senegalensis* than for *S. solea*. However, no significant differences were detected in *S. senegalensis* from the two nurseries. Larger variations in salinity (10‰ amplitude in the uppermost nursery vs 0.2‰ in the lower nursery) and highest pollution loads may be important factors lowering the habitat quality of the uppermost nursery in comparison to the lower nursery. The use of growth rate estimates based on otolith readings and the RNA / DNA index as tools for habitat quality assessment was discussed.

Introduction

Growth and survival in early life stages strongly influence successful recruitment to the adult populations (Houde, 1987; Van der Veer et al., 1990). Rapid growth means that less time is spent in the most vulnerable size ranges and that larger individuals will prevail by the end of the nursery period, along with the related competitive advantages (Van der Veer and Bergman, 1987; Sogard, 1992, 1997; Ellis and Gibson, 1995).

Fish nurseries are often found in estuaries and shallow coastal waters, which provide suitable conditions for survival and enhancement of growth, namely high food abundance, refuge from predators and higher water temperature (Haedrich, 1983; Miller et al., 1991; Beck et al., 2001). Assessing habitat quality of nursery areas has been a long pursued and difficult goal for estuarine and marine biologists due to many interacting factors (e.g. Sustainable Fisheries Act, US Senate, 1996; Brown et al., 2000; Eastwood et al., 2003; Le Pape et al., 2003). The recent European Water Framework Directive (2000 / 60 / EC; EC (European Communities), 2000) follows a similar philosophy, concentrating on the need for identification of good ecological standards for the protection of specific water bodies (e.g. estuaries).

The estimation of habitat specific growth rates is a key step for the determination of habitat quality (Able et al., 1999). Growth rates based on otolith daily rings provide an accurate measure of growth that integrates the whole life of the fish.

Nucleic acid quantification and subsequent RNA-DNA ratios has been used in numerous studies as indices for nutritional condition and growth assessment in larvae and juvenile fish (e.g. Buckley, 1984; Richard et al., 1991; Gwack and Tanaka, 2001). This biochemical index reflects variations in growth related protein synthesis, since RNA concentration fluctuates both with food intake and protein requirement, while DNA somatic content remains constant, providing a recent picture of overall fish condition and growth (Buckley and Bullow, 1987; Bullow, 1987).

Various studies have assessed habitat quality and compared different sites. Habitat quality differences have been found along pollution gradients (Burke et al., 1993), in areas impacted by man-made structures (Able et al., 1999), in protected marine reserves (Lloret and Planes, 2003), and between estuarine and nearshore flatfish nurseries (Yamashita et al., 2003; Gilliers et al., 2004).

The Tagus estuary has been used as a nursery area by two commercially important species of sole, the common sole *Solea solea* (Linnaeus, 1758) and the Senegal sole, *Solea senegalensis* Kaup 1858 (Costa and Bruxelas, 1989; Cabral and Costa, 1999). Two specific nursery areas have been identified within the estuary, one in the uppermost section that is used by juveniles of both species, and another in the upper eastern section (also in the upper estuary but at a lower location), used only by *S. senegalensis* (Costa and Bruxelas, 1989; Cabral and Costa, 1999). Niche overlap has been reported, albeit for a short period (Cabral, 2000).

Both Cabral (2003) and Fonseca et al. (2006) investigated growth for the Tagus estuary soles using length frequency progression methods, yet pointed out the limitations of these methods and called for the application of a more accurate growth rate determination method. These authors reported higher growth rates for soles in the Tagus estuary than in other important North-European nurseries (although they did not discriminate the two nursery areas of the Tagus in their analyses). Fonseca et al. (2006) aimed at evaluating monthly variation in the condition of the various cohorts colonizing the

estuary throughout time and concluded that RNA / DNA variation patterns over the nursery period reflected growth and estuarine colonization patterns.

While *S. solea* is a temperate species with a distribution that ranges from the Baltic Sea to Senegal, *S. senegalensis* is a tropical species that ranges from South Africa to the Bay of Biscay (Quéro et al., 1986). The Tagus estuary is one of the few nurseries where both sole species are present in high abundance (Cabral and Costa, 1999).

Studies on *S. senegalensis* ecology are scarce (Dinis, 1986; Andrade, 1992; Cabral and Costa, 1999; Cabral, 2000, 2003; Anguis and Cañavate, 2005) and do not allow for conclusive remarks about recruitment variability, while for *S. solea* an important body of literature has already been developed. It is generally agreed that recruitment of *S. solea* is determined before the end of the first year of life, and that water temperature plays an important role (e.g. Rijnsdorp et al., 1992; Wegner et al., 2003; Henderson and Seaby, 2005). However, most studies were conducted in temperate waters. Understanding the role of habitat quality in the early life of fish over its full range of distribution is very important for essential fish habitat determination, particularly for species such as the soles that are the main target of fisheries over a wide geographical area.

The present paper aims at: (i) estimating habitat specific growth rates and condition indices in *S. solea* and *S. senegalensis*, in two nursery areas of the Tagus estuary (Portugal) based on otolith daily rings and RNA-DNA ratio, respectively; and at (ii) discussing the use of both methodologies as tools for habitat quality monitoring of the nursery grounds for the sole species concerned.

Materials and methods

Study areas

The Tagus estuary (Fig. 1) is one of the largest estuaries in Western Europe (325 km^2). It is a partially mixed estuary with a mean tidal range of about 4 m. Approximately 40% of the estuarine area is intertidal. Much of its upper area is composed by extensive intertidal mudflats fringed by saltmarshes (Caçador and Vale, 2001). Two important sole nurseries were identified in the Tagus estuary in previous studies (A, Vila Franca de Xira, and B, Alcochete; Fig. 1) by Costa and Bruxelas (1989) and Cabral and Costa (1999). Although most of the environmental factors present a wide and similar range in these two areas, some differences can be outlined. The uppermost area, A, is deeper (mean depth 4.4 m), presents lower and highly variable salinity and has a higher proportion of fine sand in the bottom substrate. Nursery area B is shallower (mean depth 1.9 m), and more saline, with lower variability in salinity, while the bottom substrate is mainly composed of mud (Cabral, 1998; Cabral and Costa, 1999). Nursery A is located in an industrialised area that receives substantial quantities of industrial and urban sewage, while nursery B is located in an area which is under much lower human pressure and has no important adjacent industries (Vale, 1986).

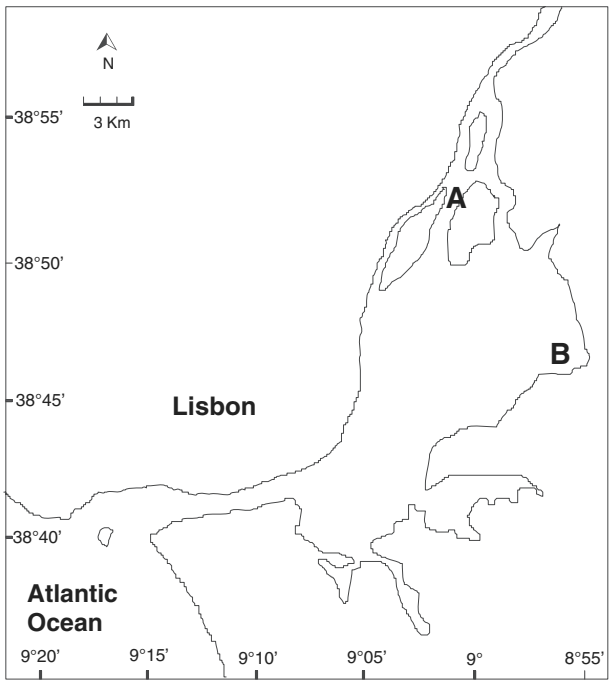

Fig. 1. Location of nursery areas (A and B) within Tagus estuary

Climate in this area is Mediterranean with mild winters and warm and dry summers (Aschmann, 1973).

Juvenile collections

Both nurseries were surveyed monthly from March to October 2005 in order to determine the beginning and the end of estuarine colonization by 0-group juvenile soles. From late June (when the first 0-group juveniles were detected in the nursery areas) and during July (when colonization ended) surveys were intensified, taking place at approx. two-week intervals, in order to better determine the end of the estuarine immigration process of the first cohort of each species.

S. solea is a temperate species with a temporally restricted spawning period leading to a estuarine colonization concentrated in time. *S. senegalensis*, however, has a very wide spawning period (Anguis and Cañavate, 2005) which is characteristic of tropical species and leads to several successive cohorts. Cabral (2003) and Fonseca et al. (2006) observed that growth and condition is higher for the first cohort of both species entering the estuary, indicating that direct comparisons should take into account the estuarine colonization process. In 2005, the first cohort of both species occurred at approx. the same time, presenting the highest densities when compared to subsequent cohorts. In order to work with comparable samples containing enough numbers of individuals for growth and condition assessment, we chose to study the first cohort of each species.

Length frequency of the first cohorts of 0-group juveniles was analysed at the end of the colonization period for each nursery area and for each species (to insure that the whole cohort had already arrived at the nursery) (Fig. 2). Age and condition were determined in 0-group *S. solea* and *S. senegalensis* collected at eight stations in nursery A and in 0-group *S. senegalensis* collected at six stations in nursery B (at the end of the estuarine colonization). Trawls were conducted with a 2.5 m beam trawl with 5 mm stretched mesh at the codend.

All samples were frozen immediately after collection. In the laboratory individuals were identified, counted and their total length measured to the nearest mm.

Environmental data

During each trawl environmental data, such as water temperature and salinity, were registered with a multiparameter

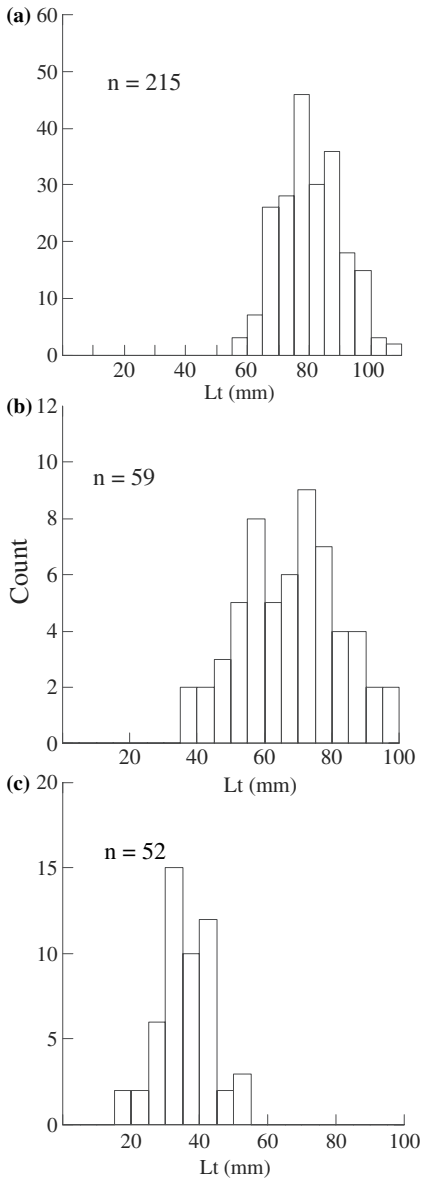

Fig. 2. Length-frequency distribution of 0-group soles caught in Tagus estuary, July 2005: (a) *S. solea,* nursery A; (b) *S. senegalensis*, nursery A; c) *S. senegalensis*, nursery B

probe, from March to October 2005. Environmental data were statistically explored with SYSTAT 10.0. For the above-stated reasons (comparability of samples) we focused on the June–July period and compared only the first cohort of juveniles. Mean values and standard deviations were estimated for water temperature and salinity in both nursery areas during the June–July period.

Growth rate estimation

Otoliths of a subsample of juveniles chosen randomly from each length category (5 mm length categories) were examined. The left lapillus, which has the longer axis due to the bilateral asymmetry between the right and left lapillus, was used for all age estimates. Lapillar otoliths were used because they are relatively thin and have well-defined increments that are spatially more uniform than the sagittae otoliths which have accessory primordia (Amara et al., 1994). Otoliths were removed and mounted with glue on microscope slides and polished in the sagital plane to the central primordial with an aluminium oxide polishing bar.

Otoliths were analysed under transmitted light at 400× or 1000× magnification, using a video system fitted to a compound microscope. Otolith counts were made along the posterior axis. Otolith increments were counted three times (by the same reader); the age was regarded as the mean of the three counts. Precision was estimated by computing the coefficient of variation. Otoliths were eliminated whenever the reading variation was above 5%.

Age was estimated for 151 *S. solea* and 59 *S. senegalensis* from nursery A, and for 52 *S. senegalensis* from nursery B.

Growth was described by a linear model. An analysis of covariance (ANCOVA) was conducted to test differences in growth between nursery areas and species (slope of age against length).

RNA-DNA ratio determination

Nucleic acid determination was carried out following the fluorometric method described by Caldarone et al. (2001) and adapted to a cuvette spectrofluorometer, as described in Fonseca et al. (2006). Detection limits, standard calibration curves for RNA, DNA and spike recovery of homogenate samples (n = 3) were first determined with a series of dilutions of pure calf-thymus DNA (Calbiochem) and 18S- and 28S-rRNA (Sigma). Tissue sample autofluorescence and residual fluorescence were analysed, the latter by adding 1 U μl^{-1} DNase (n = 3) (Sigma). Concentrations of stock standard RNA and DNA solutions were first checked with an UV-spectrophotometer.

To ensure reproducibility, two 20 mg (dry weight) replicates of each juvenile sole were analysed. White muscle was homogenised through short term ice-sonication with 200 μl of 1% sarcosine solution (*N*-lauroylsarcosine), and then diluted with 1.8 ml Tris-EDTA buffer (Trizma, pH 7.5) (sarcosine final concentration of 0.1%). Total nucleic acid fluorescence (RNA and DNA) was measured by adding 300 μl sample homogenate, 1.8 ml Tris-EDTA and 150 μl Ethidium Bromide (EB, 1 mg ml^{-1}) to the first vial. DNA fluorescence was determined by digesting RNA content with 150 μl RNase (A from bovine pancreas, 20 U ml^{-1} incubated at 37°C for 30 min, Sigma) in the second vial containing 300 μl sample homogenate, 1.65 ml Tris-EDTA and 150 μl EB. Excitation and emission wavelengths used were 360 nm and 600 nm, respectively. RNA fluorescence value was determined by subtracting the DNA fluorescence reading (second reading) from the total fluorescence value (first reading). RNA and DNA content in tissue samples was calculated through calibration curves previously constructed plus the dilution factors used.

T-tests were performed in order to compare conditions between the two nursery areas, and between both species. Interspecific comparison is generally not carried out, as RNA-DNA ratio is species-specific (Bullow, 1987). Yet, *S. solea* and *S. senegalensis* are genetically very closely related and are thus regarded as sister-species (Ben-Tuvia, 1990; Tinti and Picinetti, 2000); thus, we found that between-species comparison of this condition index was both interesting and justified. Since the RNA-DNA ratio is dependent on age of the individual, tests were performed only between overlapping length ranges. Comparisons were made between both species at nursery A and between *S. senegalensis* from nursery A and B. The software STATISTICA was used for the test procedures.

Results

Estuarine colonization by soles in 2005

The first cohorts of both soles colonized the estuary in June–July, establishing spatial and temporal sympatry in the upper nursery area, but not in the lower nursery where only *S. senegalensis* was present, as previously observed (Cabral and Costa, 1999; Cabral, 2003). As expected, the first cohort of *S. senegalensis* was followed by new cohorts entering the estuary in the following months. *S. solea* presented only one cohort.

Environmental conditions during the study period

In the June–July period, mean salinity in nursery A was 12.9‰ (SD = 3.0; minimum = 6.9‰; maximum = 16.9‰; n = 16), while in nursery B it was 32.5‰ (SD = 0.1; minimum = 32.4‰; maximum = 32.6‰; n = 12). Mean water temperature in nursery A was 24.4°C (SD = 0.9; minimum = 23.5°C; maximum = 25.7°C), while in nursery B it was 25.0°C (SD = 0.5; minimum = 24.3°C; maximum = 25.9°C).

Length frequency, growth and condition of the juveniles

Length frequency distribution of 0-group juveniles at the end of the colonization period showed approx. normal distributions for both species and nurseries studied (Fig. 2).

Growth during the first months following settlement was best described by a linear model (Fig. 3). *S. solea* 0-group juveniles growth rate was estimated to be 0.767 mm per day (range of total length of individuals analysed, TL : 57–109 mm; n = 215) in nursery A. *S. senegalensis* 0-group juveniles growth rate was estimated as 0.970 mm per day (range of total length of individuals analysed, TL : 36–99 mm; n = 59) in nursery A, while in nursery B growth rate was estimated as 1.180 mm per day (range of total length of individuals analysed, TL : 19–52 mm; n = 52). Thus, *S. solea* had a slower growth rate than *S. senegalensis* in both nurseries ($P < 0.05$), while *S. senegalensis* from nursery B presented the fastest rate ($P > 0.05$).

Mean RNA-DNA ratio was 2.90 for *S. solea* (nursery A), while for *S. senegalensis* 3.50 in nursery A and 4.01 in nursery B. Condition was significantly different between the two species in nursery A (t test = −3.81, $P < 0.05$), while no significant differences were detected between *S. senegalensis* from nursery A and B ($t = 0.25$, $P > 0.05$).

Condition peaked in the second length class in both species from nursery A, while in nursery B peak condition was observed in the third length class (Fig. 4). After reaching a peak, RNA-DNA ratio declined with fish length in both species. The smaller category lengths presented low values, especially in *S. senegalensis* from nursery B.

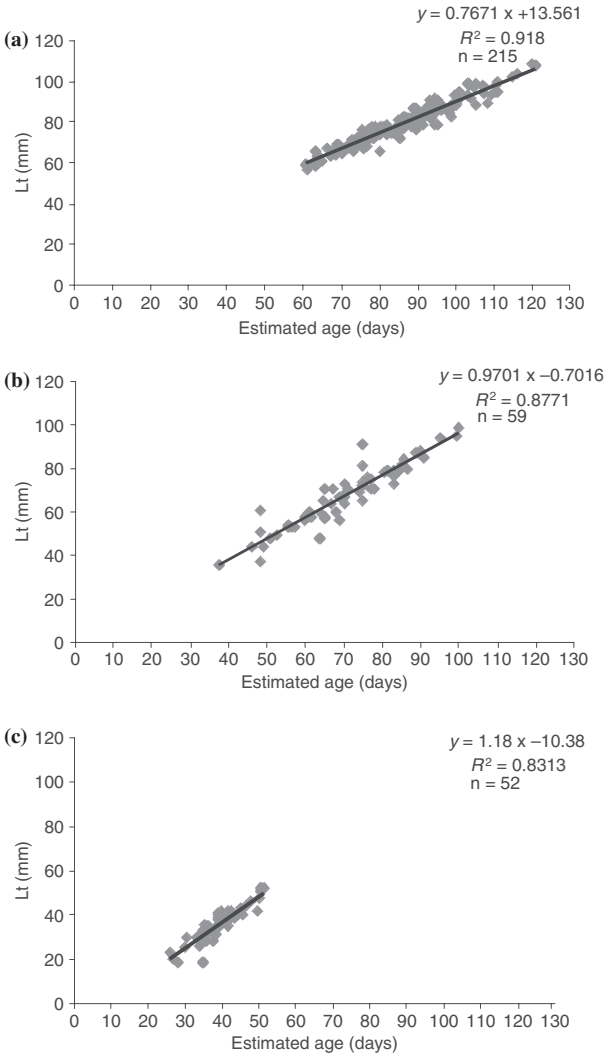

Fig. 3. Regression of soles total length (mm) against estimated age (days) by daily otolith increments: (a) *S. solea*, nursery A; (b) *S. senegalensis*, nursery A; (c) *S. senegalensis*, nursery B

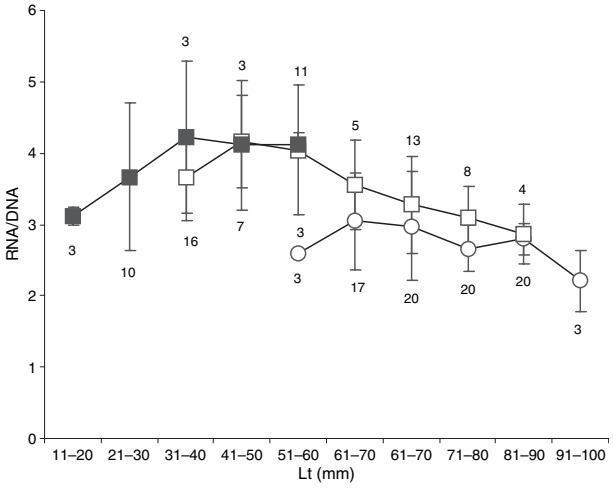

Fig. 4. RNA-DNA mean ratios [vertical bars represent standard deviations; n is given at the top (*S. senegalensis,* nursery A) or bottom (*S. senegalensis*, nursery B; *S. solea*, nursery A) of each SD bar] for 0-group *S. solea*, nursery A –◇–; for *S. senegalensis*, nursery A –□– and for *S. senegalensis*, nursery B –■–. Lines connecting mean values were not calculated

Discussion

Habitat specific growth rates estimated through otolith readings revealed differences between nurseries and sole species, while habitat specific condition based on RNA-DNA ratio revealed differences between species but not between nurseries.

Higher growth rates were found in *S. senegalensis* from nursery B than from nursery A. RNA-DNA ratios did not reveal any differences between nursery areas, but were higher for *S. senegalensis* than for *S. solea*, both inhabiting nursery A. Interspecific comparison of growth rates within nursery A also

revealed a higher value for *S. senegalensis*. These results seem to indicate that nursery B has a higher quality of habitat than nursery A.

One important issue regarding differences among nurseries is cross migration of juveniles between the two areas. Vinagre et al. (2007) concluded that Tagus estuary 0-group soles exhibit high site fidelity to the nursery they colonize, meaning that migration should not be a confounding factor in growth rates and condition estimates carried out in the present study.

Differences between nursery areas depend on multiple factors not always clearly identifiable due to the highly complex and variable nature of estuarine systems. Yet, differences between the sole nurseries in the Tagus estuary are possibly related to salinity, prey availability and pollution levels. Nursery B has more stable salinity levels than nursery A, implying that an important amount of energy that would be used for constant adjustment to salinity variation can be diverted to growth (Evans, 1993; Moyle and Cech, 1996). Prey availability is also different between the two areas. While the main prey in nursery A is *Corophium* spp., in nursery B *Scrobicularia plana* (da Costa, 1778) is the principal prey (Cabral, 1998). The higher calorific content of bivalves compared to amphipods is possibly an important factor determining growth rates (Cummins and Wuycheck, 1971). Other important aspects differentiating nursery A from B are pollution load and human pressure (Vale, 1986). The lower pollution stress levels to which fish are exposed in nursery B should be important for general health and growth.

Growth rates for *S. solea* were higher in the Tagus than in northern European nursery areas (e.g. Rogers, 1994; Jager et al., 1995; Amara et al., 2001; Amara, 2004). This was also reported by Cabral (2003) and Fonseca et al. (2006) using modal progression analysis of length-frequency data. Higher growth rates are to be expected in southern Europe due to higher water temperatures (Yamashita et al., 2003; Henderson and Seaby, 2005) as well as longer photoperiods throughout the year (Devauchelle et al., 1987; Boeuf and Le Bail, 1999).

S. senegalensis growth rates were higher than those reported by Andrade (1992) in the Ria Formosa and Cabral (2003) in the Tagus estuary but similar to those reported by Fonseca et al. (2006) for the first cohort of this species in the Tagus estuary.

RNA-DNA ratios for juvenile soles were within the range of other studies on juvenile flatfishes (−1.1–8.2) (e.g. Mathers et al., 1992; Yamashita et al., 2003; Gilliers et al., 2004). Experiments with reared *S. solea* concluded that RNA-DNA ratio of fed fish was around 2 (Richard et al., 1991). Richard et al. (1991) pointed out that indices from reared and wild fish must be compared with caution, since food offered to captive fish may be of lower nutritional value than wild prey. Keeping this important issue in mind, it can be concluded that soles from the Tagus estuary were of fairly good nutritional status.

As reported by other authors, the RNA-DNA ratio was found to be dependent on age (Buckley and Bullow, 1987; Buckley et al., 1999). A distinctive pattern of decreasing RNA-DNA ratio with fish length was observed for both species in nursery A, but not in nursery B. Lower condition values were noticeable in the first length classes for both species and nursery areas, especially evident in nursery B. Since the RNA-DNA ratio reflects recent growth, this could be due to temporarily unfavourable conditions that affected the smaller individuals of both nurseries and species.

The higher growth rates and condition of *S. senegalensis* when compared to *S. solea* can have important implications in a warming climate scenario. *S. senegalensis* appears to be better adapted than *S. solea* to the present environmental conditions of the Tagus estuary. Temperature is one of the most important factors determining growth; the Tagus has higher temperatures than the northern European estuaries where *S. solea* thrives but where *S. senegalensis* is not present. Water temperature in the upper Tagus estuary is usually above 23°C during the summer months, well above the *S. solea* metabolic optimum temperature of approx. 19°C (LeFrançois and Claireaux, 2003). *S. senegalensis* metabolic optimum temperature has not yet been determined, but being a subtropical species it will probably be higher than that of *S. solea*. Also, spawning, egg incubation and rearing temperatures for *S. senegalensis* are considerably higher than for *S. solea* (Imsland et al., 2003). Thus in a warming climate scenario, lower densities of temperate species such as *S. solea* and higher densities of subtropical species such as *S. senegalensis* are to be expected, as noted by Cabral et al. (2001).

Both methods used in the present study provided valuable information concerning habitat quality. While growth rates estimated from daily otolith rings provide long-term information on growth throughout the entire life of the fish, RNA-DNA ratios provide information only on recent growth, around 1 week for juvenile fish (Richard et al., 1991). Thus growth rates based on otolith readings are influenced not only by habitat quality of preceding months in the nursery, but also by the marine environment prior to immigration to the nursery areas. This may be a limitation when the objective is to estimate habitat quality solely in an estuarine nursery area. Recent growth assessed through RNA-DNA ratios is quite valuable, as it is based solely on the conditions provided by the nursery area, yet it can be influenced by unusual events that do not reflect the average habitat quality of the area. Intensive sampling for RNA-DNA ratios determination starting at the beginning of the estuarine colonization could yield very interesting results; however, as this index only reflects the nutritional condition of the fish over short periods, assessment of habitat quality over a period of ca. 2 months would be quite costly and time consuming. The combination of both indices used in this study integrating habitat quality over a long period with recent condition is quite interesting for habitat quality determination in highly variable environments such as estuarine nurseries, since the information given by both methods complement each other.

Other methods such as recent growth estimation based on marginal otolith increment width (e.g. Amara and Galois, 2004; Gilliers et al., 2004), condition based on protein concentration (e.g. Peragón et al., 2001; Weber et al., 2003), condition based on lipid content (e.g. Galois et al., 1990; Lloret and Planes, 2003) and the use of molecular biomarkers in areas subjected to pollution (e.g. Nunes et al., 2005; Rendón-von Osten et al., 2005) are also very promising. Further research will certainly determine the most appropriate combinations of indices for each species and habitat type.

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References

Able, K. W.; Manderson, J. P.; Studholme, A., 1999: Habitat quality for shallow water fishes in an urban estuary: the effects of man-made structures on growth. Mar. Ecol. Prog. Ser. **187,** 227–235.

Amara, R., 2004: 0-group flatfish growth conditions on a nursery ground (Bay of Canche, Eastern English Channel). Hydrobiologia **518,** 23–32.

Amara, R.; Galois, R., 2004: Nutritional condition of metamorphosing sole: spatial and temporal analyses. J. Fish Biol. **64,** 72–88.

Amara, R.; Desaunay, Y.; Lagardère, F., 1994: Seasonal variation in growth of larval sole *Solea solea* (L.) and consequences on the success of larval immigration. Neth. J. Sea Res. **32,** 287–298.

Amara, R.; Laffargue, P.; Dewarumez, J. M.; Maryniak, C.; Lagardère, F.; Luczac, C., 2001: Feeding ecology and growth of 0-group flatfish (sole, dab and plaice) on a nursery ground (Southern Bight of the North Sea). J. Fish Biol. **58,** 788–803.

Andrade, J. P., 1992: Age, growth and population structure of *Solea senegalensis* Kaup 1858, (Pisces, Soleidae) in the Ria Formosa (Algarve, Portugal). Sci. Mar. **56,** 35–41.

Anguis, V.; Cañavate, J. P., 2005: Spawning of captive Senegal sole (*Solea senegalensis*) under a naturally fluctuating temperature regime. Aquaculture **243,** 133–145.

Aschmann, H., 1973: Distribution and peculiarity of Mediterranean ecosystems. Origins and structure. In: Ecological studies. F. Castri and H. A. Mooney (Eds). Springer-Verlag, Berlin, pp. 11–19.

Beck, M.; Heck, K.; Able, K.; Childers, D.; Egglestone, D.; Gillanders, B.; Halpern, B.; Hays, C.; Hoshino, K.; Minello, T.; Orth, R.; Sheridan, P.; Weinstein, M., 2001: The identification, conservation and management of estuarine and marine nurseries for fish and invertebrates. Bioscience **51,** 633–641.

Ben-Tuvia, A., 1990: A taxonomic reappraisal of the Atlanto-Mediterranean soles *Solea solea*, *Solea senegalensis* and *Solea lascaris*. J. Fish Biol. **36,** 947–960.

Boeuf, G.; Le Bail, P.-Y., 1999: Does light have an influence on fish growth? Aquaculture **177,** 129–152.

Brown, S.; Buja, K.; Jury, S.; Monaco, M.; Banner, A., 2000: Habitat suitability index models for eight fish and invertebrate species in Casco and Sheepscot bays, Maine N. Am. J. Fish. Manag. **20,** 408–435.

Buckley, L. J., 1984: RNA-DNA ratio: an index of larval fish growth in the sea. Mar. Biol. **80,** 291–298.

Buckley, L. J.; Bullow, F. J., 1987: Techniques for estimation of RNA, DNA and protein in fish. In: Age and growth of fish. R. C. Summerfelt and G. E. Hall (Eds), Iowa State Univ. Press, Ames, Iowa, pp. 345–354.

Buckley, L. J.; Caldarone, E.; Ong, T. L., 1999: RNA-DNA ratio and other nucleic acid based indicators for growth and condition of marine fishes. Hydrobiologia **401,** 265–277.

Bullow, F. J., 1987: RNA-DNA ratios as indicators of growth in fish: a review. In: Age and growth of fish. R. C. Summerfelt and G. E. Hall (Eds). Iowa State University Press, Ames, Iowa, pp. 45–64.

Burke, J. S.; Peters, D. S.; Hanson, P. J., 1993: Morphological indices and otolith microstructure of Atlantic croacker. *Micropogonias undulatus*, as indicators of habitat quality along an estuarine pollution gradient. Environmental Biology of Fishes **36,** 25–33.

Cabral, H. N., 1998: Utilização do estuário do Tejo como área de viveiro pelos linguados, *Solea solea* (L., 1758) e *Solea senegalensis* Kaup, 1858, e robalo, *Dicentrarchus labrax* (L., 1758). PhD Thesis, Universidade de Lisboa, Lisboa, Portugal.

Cabral, H. N., 2000: Comparative feeding ecology of sympatric *Solea solea* and *Solea senegalensis* within the nursery areas of the Tagus estuary, Portugal. J. Fish Biol. **57,** 1550–1562.

Cabral, H. N., 2003: Differences in growth rates of juvenile *Solea solea* and *Solea senegalensis* in the Tagus estuary, Portugal. J. Mar. Biol. Assoc. U.K. **83,** 861–868.

Cabral, H. N.; Costa, M. J., 1999: Differential use of nursery areas within the Tagus estuary by the sympatric soles, *Solea solea* and *Solea senegalensis*. Environ. Biol. Fish **56,** 389–397.

Cabral, H. N.; Costa, M. J.; Salgado, J. P., 2001: Does the Tagus estuary fish community reflect environmental changes? Climate Res. **18,** 119–126.

Caçador, I.; Vale, C., 2001: Salt marshes. In: Metals in the environment. M. N. V. Prasad (Ed.). University of Hyderabad, Hyderabad, pp. 99–116.

Caldarone, E. M.; Wagner, M.; St. Onge-Burns, J.; Buckley, L. J. 2001: Protocol and guide for estimating nucleic acids in larval fish using a fluorescence microplate reader. Northeast Fisheries Science Center, Ref. Doc 01-11 p 1–22. National Marine Fisheries Service, Woods Hole, MA.

Costa, M. J.; Bruxelas, A., 1989: The structure of fish communities in the Tagus estuary, Portugal, and its role as a nursery for commercial fish species. Sci. Mar. **53,** 561–566.

Cummins, K. W.; Wuycheck, J. C., 1971: Caloric equivalents for investigations in ecological energetics. Internationale Vereinigung für Theoretische und Angewandte Limnologie 18, Mitteilung 158.

Devauchelle, N.; Alexandre, J. C.; Le Corre, N.; Letty, Y., 1987: Spawning of sole (*Solea solea*) in captivity. Aquaculture **66,** 125–147.

Dinis, M.T., 1986: Quatre soleidae de l'estuaire du Tage. Reproduction et croissance. Essai d'élevage de *Solea senegalensis* Kaup. PhD Thesis, Université de Bretagne Occidentale, Brest, France.

Eastwood, P. D.; Meaden, G. J.; Carpentier, A.; Rogers, S. I., 2003: Estimating limits to the spatial extent and suitability of sole (*Solea solea*) nursery grounds in the Dover Strait. J. Sea Res. **50,** 151–165.

EC (European Communities), 2000: Directive 2000 / 60 / EC of the European Parliament and of the Council of 23rd October 2000, establishing a framework for community action in the field of water policy. Official J. Eur. Commun. L **327,** 1–72.

Ellis, T.; Gibson, R. N., 1995: Size selective predation of 0-group flatfishes on a Scottish coastal nursery ground. Mar. Ecol. Prog. Ser. **127,** 27–37.

Evans, D. H., 1993: Osmotic and ionic regulation. In: The physiology of fishes. D. H. Evans (Ed.). CRC Press, Boca Raton, FL, pp. 315–341.

Fonseca, V. F.; Vinagre, C.; Cabral, H., 2006: Growth variability of juvenile sole *Solea solea* (Linnaeus, 1758) and *Solea senegalensis* Kaup, 1858, and comparison with RNA-DNA ratios in the Tagus estuary, Portugal. J. Fish Biol. **68,** 1551–1562.

Galois, R.; Lagardère, F.; Richard, P., 1990: Changes in biochemical composition and otolith microstructure of larval common sole, *Solea solea* (L.) under experimental starvation. La Mer **28,** 273–285.

Gilliers, C.; Amara, R.; Bergeron, J. -P.; Le Pape, O., 2004: Comparison of growth and condition indices on juvenile flatfish in different coastal nursery grounds. Environ. Biol. Fish **71,** 189–198.

Gwack, W. S.; Tanaka, M., 2001: Developmental changes in RNA:DNA ratios of fed and starved laboratory-reared Japanese flounder larvae and juveniles, and its application to assessment of nutritional condition for wild fish. J. Fish Biol. **59,** 902–915.

Haedrich, R. L., 1983: Estuarine fishes. In: Ecosystems of the World 26 Estuarine and Enclosed Seas. B. Ketchum (Ed.). Elsevier, Amsterdam, pp. 183–207.

Henderson, P. A.; Seaby, R. M., 2005: The role of climate in determining the temporal variation in abundance, recruitment and growth of sole *Solea solea* in the Bristol Channel. J. Mar. Biol. Assoc. U.K. **85,** 197–204.

Houde, E. D., 1987: Fish early life dynamics and recruitment variability. Am. Fish. Soc. Symp. **2,** 17–29.

Imsland, A. K.; Foss, A.; Conceição, L. E. C.; Dinis, M. T.; Delbare, D.; Schram, E.; Kamstra, A.; Rema, P.; White, P., 2003: A review of the culture potential of *Solea solea* and *S. senegalensis*. Rev. Fish Biol. Fish **13,** 379–407.

Jager, Z.; Kleef, H. L.; Tydeman, P., 1995: Mortality and growth of 0-group flatfish in the brackish Dollard (Ems estuary, Wadden Sea). Neth. J. Sea Res. **34,** 119–129.

Le Pape, O.; Chauvet, F.; Mahévas, S.; Lazure, P.; Guérault, D.; Désaunay, Y., 2003: Quantitative description of habitat suitability for the juvenile common sole (*Solea solea*, L.) in the Bay of Biscay (France) and the contribution of different habitats to the adult population. J. Sea Res. **50,** 139–149.

LeFrançois, C.; Claireaux, G., 2003: Influence of ambient oxygenation and temperature on metabolic scope and scope for heart rate in the common sole, *Solea solea*. Mar. Ecol. Prog. Ser. **259,** 273–284.

Lloret, J.; Planes, S., 2003: Condition, feeding and reproductive potential of white sea bream (*Diplodus sargus*) as indicators of habitat quality and the effect of reserve protection in the northwestern Mediterranean. Mar. Ecol. Prog. Ser. **248,** 197–208.

Mathers, E. M.; Houlihan, D. F.; Cunningham, M. J., 1992: Nucleic acid concentrations and enzyme activities as correlates of growth rate of the Saithe *Pollachius virens*: growth rate estimates of open sea fish. Marine Biology **112,** 363–369.

Miller, J. M.; Burke, J. S.; Fitzhugh, G. R., 1991: Early life history patterns of Atlantic North American flatfish: likely (and unlikely) factors controlling recruitment. Neth. J. Sea Res. **27,** 261–275.

Moyle, P. B.; Cech, J. J., Jr., 1996: Fishes, an introduction to Ichthyology, 3rd edn. Prentice Hall, NJ, 590 pp.

Nunes, B.; Carvalho, F.; Guilhermino, L., 2005: Acute toxicity of widely used pharmaceuticals in aquatic species: *Gambusia holbrooki*, *Artemia parthenogenica*, *Tetraselmis chuii*. Ecotoxicol. Environ. Saf. **61,** 413–419.

Peragón, J.; Barroso, J. B.; Garcia-Salguero, L.; de la Higuera, M.; Lupiañez, J. A., 2001: Growth, protein turnover rate and nucleic acid concentration in the white muscle of rainbow trout during development. Int. J. Biochem. Cell Biol. **33,** 1227–1238.

Quéro, J.-C.; Dessouter, M.; Lagardère, F. 1986: Soleidae. In: Fishes of the North-eastern Atlantic and the Mediterranean, **Vol. III**. P. Whitehead**,** M.-L. Bauchot, J.-C. Hureau, J. Nielsen and E. Tortonese (Eds). Bungay, The Chausser Press Ltd, pp. 1308–1324.

Rendón-von Osten, J.; Ortíz-Arana, A.; Guilhermino, L.; Soares, A. M. V. M., 2005: In vivo evaluation of three biomarkers in mosquitofish (*Gambusia yacatana*) exposed to pesticides. Chemosphere **58,** 627–636.

Richard, P.; Bergeron, J. P.; Boulhic, M.; Galois, R.; Ruyet, J., 1991: Effect of starvation on RNA, DNA and protein content of laboratory reared larvae and juveniles of *Solea solea*. Mar. Ecol. Prog. Ser. **72,** 69–77.

Rijnsdorp, A. D.; Van Beek, F. A.; Flatmann, S.; Millner, R. M.; Riley, J. D.; Giret, M.; De Clerck, R., 1992: Recruitment in sole stocks, *Solea solea* (L.) in the northeast Atlantic. Neth. J. Sea Res. **29,** 173–192.

Rogers, S. I., 1994: Population density and growth rate of juvenile sole *Solea solea* (L.). Neth. J. Sea Res. **32,** 353–360.

Sogard, S. M., 1992: Variability in growth rates of juvenile fishes in different estuarine habitats. Mar. Ecol. Prog. Ser. **85,** 35–53.

Sogard, S. M., 1997: Size-selective mortality in the juvenile stage of teleost fishes: a review. Bull. Mar. Sci. **60,** 1129–1157.

Sustainable Fisheries Act, US Senate, 1996: Report of the committee on Commerce, Science and Transportation on S.39: Sustainable Fisheries Act. 104th Congress, 2nd Session. US Govt Printing Office, Washington, D.C., USA. pp. 104–276.

Tinti, F.; Picinetti, C., 2000: Molecular systematics of the Atlanto-Mediterranean *Solea* species. J. Fish Biol. **56,** 604–614.

Vale, C. 1986: Distribuição de metais e matéria particulada em suspensão no sistema estuarino do Tejo. Instituto Nacional de Investigação Pesca, Dissert. Invest. Lisboa, 153pp.

Van der Veer, H. W.; Bergman, M. J. N., 1987: Predation by crustaceans on a newly settled 0-group plaice, *Pleuronectes platessa*, population in the Western Wadden Sea. Mar. Ecol. Prog. Ser. **35,** 203–215.

Van der Veer, H. W.; Phil, L.; Bergman, M. J. N., 1990: Recruitment mechanisms in North Sea Plaice *Pleuronectes platessa*. Mar. Ecol. Prog. Ser. **64,** 1–12.

Vinagre, C.; Salgado, J.; Costa, M. J.; Cabral., H. N., 2007: Nursery fidelity, primary sources of nutrition and food web interactions of the juveniles of *Solea solea* and *S. senegalensis* in the Tagus estuary (Portugal): a stable isotope approach. Est. Coast. Shelf. Sci. doi: 10.1016/j.ecss.2007.07.006.

Weber, L. P.; Higgins, P. S.; Carlson, R. I.; Janz, D. M., 2003: Development and validation of methods for measuring multiple biochemical indices of condition in juvenile fishes. J. Fish Biol. **63,** 637–658.

Wegner, G.; Damm, U.; Purps, M., 2003: Physical influences on the stock dynamics of plaice and sole in the North Sea. Sci. Mar. **67,** 219–234.

Yamashita, Y.; Tominaga, O.; Takani, H.; Yamada, H., 2003: Comparison of growth, feeding and cortisol level in *Platichthys bicoloratus* juveniles between estuarine and nearshore nursery grounds. J. Fish Biol. **63,** 617–630.

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