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BUENIA AFFINIS
DIET
SEASONAL CHANGES
ONTOGENETIC SHIFT

ABSTRACT. – The feeding ecology of De Buen's goby *Buenia affinis* was studied on a population collected in the north-eastern Adriatic Sea (Kvarner area). The sampling was carried out by SCUBA diving from May 2003 to April 2004. The stomach contents analysis revealed that this epibenthic gobiid is a carnivorous and generalist feeder. *B. affinis* fed mostly on organisms that live on the bottom or close to it, belonging to both meiofauna and macrofauna. Despite the small size of *B. affinis*, it relied on a wide size range of prey, from copepods and prodissoconch bivalve to fish, larger crustaceans and polychaetes. The diet composition of *B. affinis* was significantly different among seasons and between small-sized and large-sized individuals.

INTRODUCTION

The introduction of SCUBA diving has recently demonstrated to be a powerful tool to study small fish in the Mediterranean Sea. Among 46 gobiid species known for the Adriatic Sea, eleven species were described or recorded for the first time in the Adriatic Sea in the last two decades (Kovačić 2005). Most of them were discovered by SCUBA diving and considered to be rare elsewhere (Kovačić 2005).

In particular, *Buenia affinis* Iljin, 1930 is an endemic Mediterranean species known only from a few published records. According to Kovačić (2002), the only positive records for *B. affinis* in the western Mediterranean Sea were those from Naples, Italy (Sanzo 1911) and from the Kvarner area, Croatia. Few other doubtful records were reported both in the western and eastern Mediterranean Sea, respectively from Banyuls, France (Zander 1982, probably misidentified as *Buenia jeffreysii* (Günther, 1867) and between Nice and Genoa (Miller 1986) and postlarval specimens from the Aegean Sea (Fage 1918). From a zoogeographical perspective, the two valid species recognised in the genus *Buenia*, *B. affinis* and *B. jeffreysii*, have a distinct distribution, occurring in the Mediterranean Sea and in the eastern Atlantic, respectively (Froese & Pauly 2007).

B. affinis is an epibenthic species, usually found on a sandy bottom, at depths of 3-25 m (Kovačić 2002). Until now, the few available data on *B. affinis* were recorded from the Adriatic Sea, and concerned body morphology (morphometrics, meristics, papillae counts, coloration) and ecology (habitat, biocenosis, fish assemblage) (Kovačić 2002). Considering the hypothesis of a misidentification (i.e. *B. jeffreysii* instead of *B. affinis*), further data on feeding habits of this species were reported from Banyuls-sur-Mer, although based on very few specimens (Zander & Berg 1984).

The present paper provides a definitive account of the feeding ecology of *B. affinis*, including diet composition, seasonal changes and ontogenetic shift of food preferences and overall feeding strategy.

MATERIAL AND METHODS

Study area and sampling: Two hundred and forty specimens of *B. affinis* were obtained from the locality Oštro in the Rijeka Bay (Fig. 1), in the northern part of the Kvarner area (45º 16' N, 14º 34' E) from May 2003 to April 2004. All fish were collected on sandy bottom between 5 and 10 m depth, using a hand net and anaesthetic quinaldine during SCUBA dives. On a monthly basis, twenty specimens were collected in a single dive of approximately 30 minutes. Details of species habitat in the sampling site are reported elsewhere (Kovačić 2002). During the
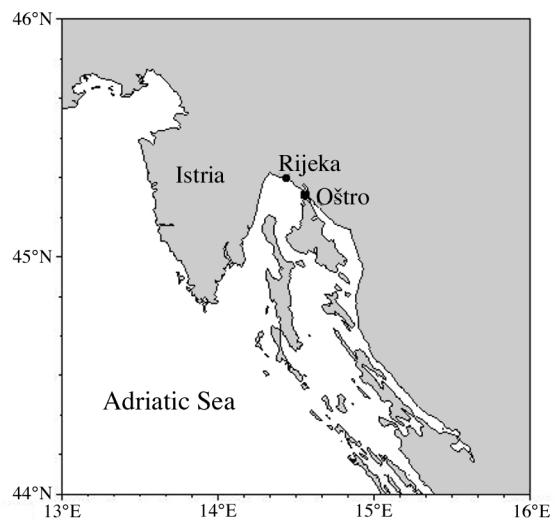

Fig. 1. – The Kvarner area, Croatia, showing sampling location Oštro of *B. affinis*.

SCUBA dives the water temperature was measured on the sea bed. All specimens were killed by over-anaesthetization with quinaldine and were stored in 65 % ethanol solution.

Laboratory methods: Total length (L_t) of all individuals was measured to the nearest 0.1 mm and wet mass (W) weighed to the nearest 0.001 g. The specimens were dissected under a stereomicroscope for the removal of gut contents. Guts were dissected and their entire content sorted to relevant taxonomic units, which were then counted. Sorted prey items and unidentifiable residue were weighed wet (W, 0.01 mg of accuracy) after blotting dry on absorbent paper. For small-sized prey, such as prodissoconch bivalves (~ 0.2 mm), halacarids, ostracods (< 1 mm) and copepods (< 1 mm) the wet mass was estimated as average value calculated on a larger sample of specimens. Wet mass of nematods was derived from published data (Baguley *et al*. 2004) because of a small total number of specimens found in the diet of material examined. Wet mass of the entire gut content (W_{GC}) was calculated as the total weight of all prey items and unidentifiable remains (Kovačić 2007).

Data analysis: The food analysis was carried out using numerical and gravimetric methods, recording number and mass of each prey item, as well as the frequency of occurrence (Berg 1979, Hyslop 1980). The frequency of occurrence was calculated taking into account for total number of fish only those with identified prey in the diet. The index of relative importance (I_{RI}), which combines the relative contribution of a food item on total stomach content by number (%N) and by mass (%W), as well as the percentage of frequency of occurrence (%O), was calculated for each prey taxon applying the following formula (Pinkas *et al*. 1971):

$I_{RI} = (\%N + \%W) \, \%O$

The feeding intensity was investigated using the fullness index (I_F) (Hureau 1970). The non-parametric Kruskal-Wallis test was used to assess seasonal differences in I_F, and the Mann-Whitney U-test was used for ontogenetic differences in I_F, as the assumptions of normality and homogeneity were not met after transformations (Sokal & Rohlf 1995). Homogeneity of variance and normality were tested with Levene's and Kolmogorov-Smirnov test, respectively. Data analyses were carried out with the SPSS 9.0 software.

The food niche breadth of the species was calculated using Levin's standardised index (B_A), that ranges between 0 and 1 (Krebs 1989):

$B_A = (n\text{-}1)^{-1}[(\Sigma_j p_j^2)^{-1}\text{-}1]$

where n is the number of prey taxa and p_j is the proportion in diet of prey j. The 95 % confidence limits of niche breadth were estimated using the jackknife method (Krebs 1989).

To assess the feeding strategy and individual contribution to niche breadth, a modification of the Costello method was applied to the prey taxa data set (Costello 1990, Amundsen *et al*. 1996). The prey-specific abundance (P_i), defined as the percentage in number of a prey taxon calculated taking into account only those predators in which the prey category actually occurs, is plotted against the frequency of occurrence (O), providing a
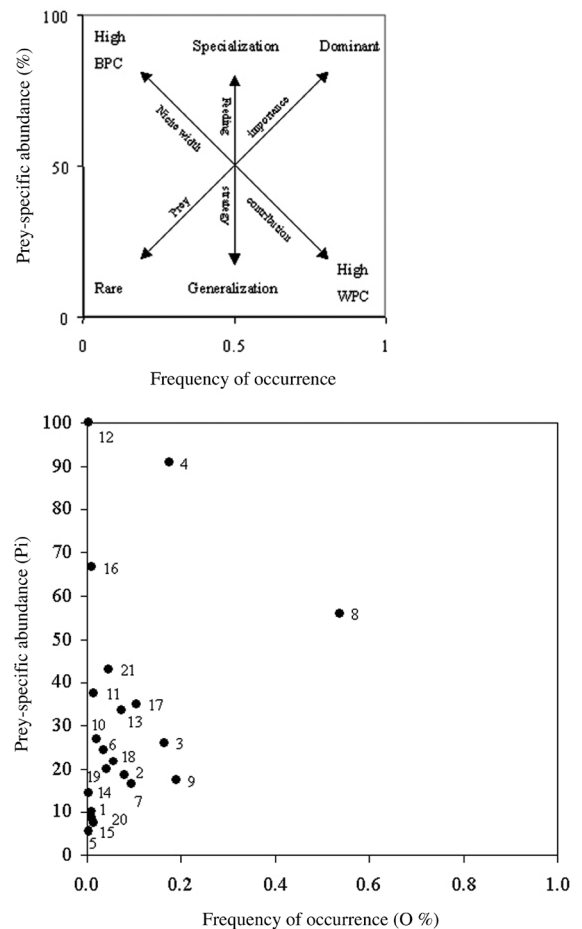

Fig. 2. – The modified Costello plot with relevant feeding axes (above). Diagram of diet composition of *B. affinis* according to Amundsen *et al*. (1996) (below). Numbers are referring to nematods (1), gastropods (2), bivalves (3), prodissoconch bivalve (4), polyplacofors (5), polychaetes (6), halacarids (7), copepods (8), ostracods (9), decapod larvae (10), natantia (11), brachyurans (12), mysids (13), cumaceans (14), anisopods (15), isopods (16), gammarids (17), caprellids (18), unidentified crustaceans (19), ophiuroids (20), fishes (21).

two-dimensional graph. The prey-specific abundance is expressed as follows:

$P_i = (\Sigma_i \, S_i / \Sigma \, S_{ti}) \, 100$

where P_i is the prey-specific abundance of prey i, S_i the total number of prey i, and S_{ti} the total number of prey in only those fish with prey i in their stomachs. The resulting plot provides information on prey importance, feeding strategy and niche width contribution inferred through the position of prey taxa in the diagram (Fig. 2). Further details can be found in Amundsen *et al*. (1996).

Seasonal changes and ontogenetic shift in diet composition were assessed using multivariate analyses of data (Marshall & Elliott 1997). For multivariate analysis, a Bray-Curtis coefficient similarity matrix was obtained from the whole data set (previously standardized and root-squared), consisting of numerical abundance of each prey taxon recorded in the stomach content of fish, excluding specimens with empty stomach. A non-metric multidimensional scaling (MDS) was applied to the pair-wise similarity matrix to order fish in a two-dimensional

plane, according to their relevant diet similarity. To assess onto-genetic shift in diet, fish were pooled in 1 mm size classes, and the mean numerical abundance of each prey taxon in each fish size class was calculated. Similarly, seasonal changes were determined pooling fish for each month of sampling. To determine relevant contributions of each prey taxon to fish distribution in the two-dimensional plot, an analysis of dissimilarity was carried out using the SIMPER routine. Finally, a one-way statistical analysis (ANOSIM routine, test R) was performed to test the null hypothesis (i.e. no statistical difference in diet between groups). R-statistic values close to unity indicate a very different dietary composition between groups, whereas values close to 0 indicate a strong similarity. All statistical analyses were made using the PRIMER software package developed at the Plymouth Marine Laboratory (Clarke & Warwick 1994, Clarke & Gorley 2001).

RESULTS

Diet composition

The gut contents of the 240 fish (12.5 mm $\leq L_t \leq$ 38.3 mm) were examined. They contained 19 different taxa, and two different development stages for two taxa (Table I). Overall, 52 specimens had empty stomachs. Number of prey items per stomach ranged between 1 and 71 (mean 7.1; SE 0.7). I_F of the total sample was 0.60 (95 % confidence intervals 0.48-0.73). The most frequently occurring prey item (% O > 50 %) was copepods, followed by few taxa with a frequency higher than 10 % (ostracods, bivalves prodissoconch, bivalves and gammarids) (Table I). Copepods and prodissoconch bivalves were numerically overwhelmingly the most important prey taxa, together constituting more than 80% of the total number of all prey ingested; respectively, they accounted for 2478.8 I_{RI} (60.7 %) and 771.9 I_{RI} (18.9 %) (Table I). Few other taxa, such as gammarids, mysids, bivalves, fishes and ostracods (in order of decreasing importance) can be considered secondary prey, accounting for an I_{RI} value ranging between 100 and 200 (2-4 % I_{RI}) (Table I). All other taxa represented rare prey, with I_{RI} < 50 ($\leq$ 1 % I_{RI}).

Prey importance in relation to percentage mass (% W) was rather different from numerical abundance. In decreasing order of importance, fishes, mysids, gammarids, polychaetes and copepods made up about 75 % of total prey biomass (Table I). All other prey was eaten in small amounts, each of them accounting for less than 5 % W (Table I). Among them, prodissosonch bivalves yielded only 2 % W, although they represented by far the most abundant prey in number (see above).

The diet breadth of the entire sample of *Buenia* was relatively low (Levin's standardised index B_A = 0.098 (95 % confidence intervals 0.075-0.122).

Table I. – Diet composition of *B. affinis* from the Kvarner area. % N, numerical percentage; % W, weight percentage; % O, frequency of occurrence; I_{RI}, index of relative importance; % I_{RI}, I_{RI} percentage.

Prey taxa	% N	% W	% O	I_{RI}	I_{RI}%
Nematods	0.1	< 0.1	1.1	0.2	< 0.1
Gastropods	1.6	3.7	8.0	41.8	1.0
Bivalves	4.8	2.7	16.5	123.9	3.0
Bivalve larvae	41.9	2.0	17.6	771.9	18.9
Polyplacophorans	0.1	0.2	0.5	0.1	< 0.1
Polychaetes	0.6	9.1	3.7	36.2	0.9
Halacarids	1.9	0.4	9.6	21.9	0.5
Copepods	39.0	7.2	53.7	2478.8	60.7
Ostracods	4.1	1.5	19.1	107.3	2.6
Decapod larvae	0.3	0.8	2.1	2.4	0.1
Natantians	0.2	4.2	1.6	7.1	0.2
Brachyurans	0.1	0.8	0.5	0.5	< 0.1
Mysids	1.0	20.1	7.4	157.4	3.9
Cumaceans	0.1	< 0.1	0.5	0.1	< 0.1
Anisopods	0.2	0.7	1.6	1.4	< 0.1
Isopods	0.1	0.1	1.1	0.3	< 0.1
Gammarids	1.6	14.2	10.6	167.4	4.1
Caprellids	0.8	2.7	5.9	20.5	0.5
Crustaceans unid.	0.7	5.2	4.3	24.9	0.6
Ophiuroids	0.1	0.3	1.1	0.4	< 0.1
Fishes	0.7	24.1	4.8	118.5	2.9

Feeding strategy

The pattern of distribution of prey taxa within the two-dimensional modified Costello plot is shown in Fig. 2. The overall feeding strategy, defined by the position of prey taxa with respect to the vertical axis, indicated *B. affinis* as a generalist predator, relying generally on taxa with low prey-specific abundance (P_i). Most prey, indeed, was localized in the lower part of the diagram, except for prodissoconch bivalves, copepods and isopods (Fig. 2). On the other hand, brachyurans represented a particular case, as they were the sole prey in a single stomach. Considering the prey importance, represented by the diagonal axis running from the lower left to the upper right of the diagram, the diet of *B. affinis* consisted almost exclusively of rare prey taxa, which were eaten in small amounts (low % N) and occasionally (low % O). Copepods and prodissoconch bivalves were the only dominant prey taxa. Finally, based on the position of prey taxon in relation to the other diagonal axis (i.e. that running from the lower left to the upper right of diagram), the niche width contribution is largely due to a high between phenotype component (Fig. 2). Summarizing above results, *B. affinis* appeared to be a generalist predator which feeds on several rare prey taxa. On the other hand, the population consisted also of few specialized
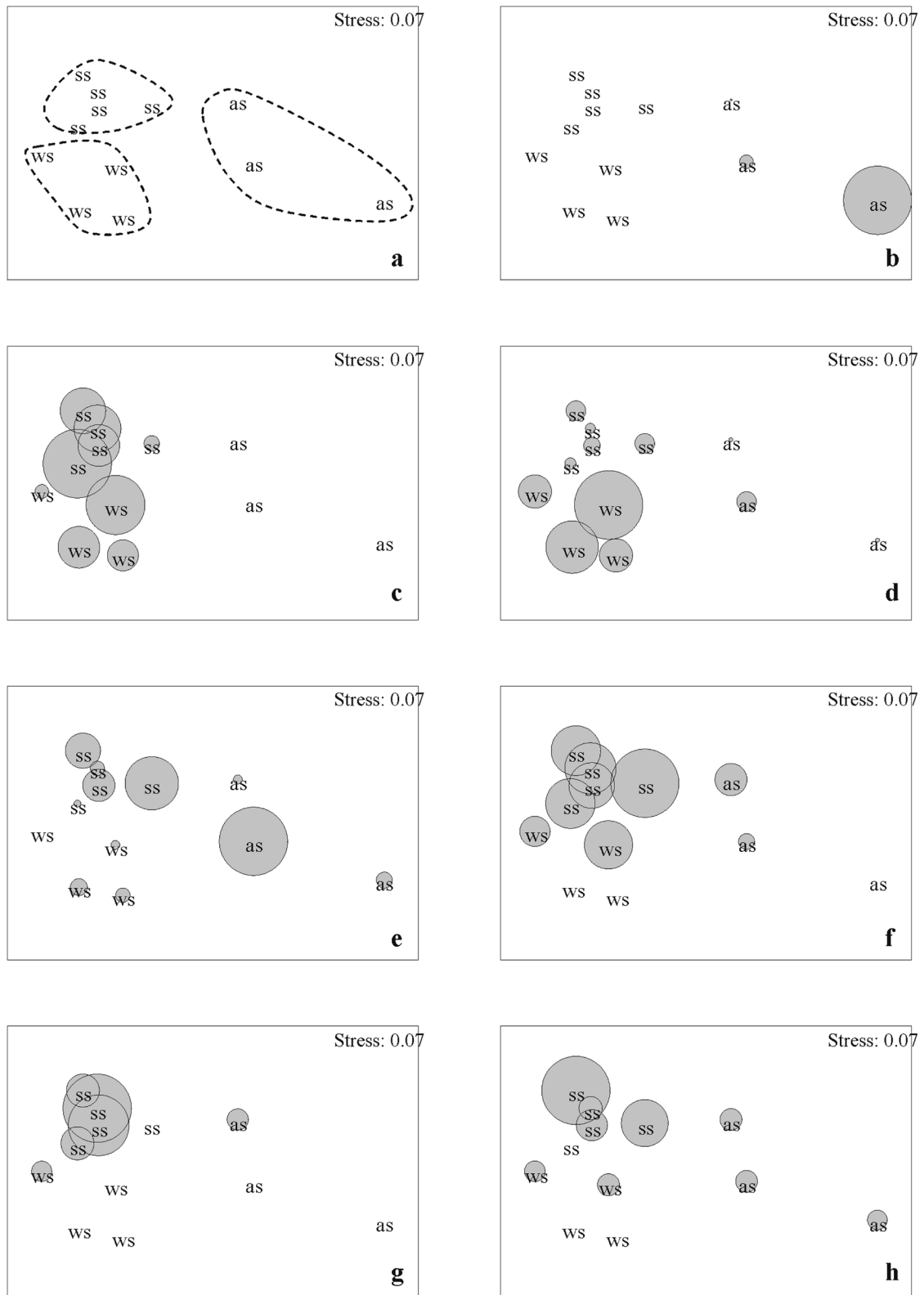
Fig. 3. – MDS analysis based on pair-wise similarity matrix derived from the mean numerical abundance of prey taxa in each sampling month. (a) MDS ordination plot of monthly samples grouped in winter season (*ws*), spring-early summer season (*ss*) and late summer-autumn season (*as*); bubble plots show mean numerical abundance pattern of the following prey taxa (superimposed to the relevant MDS plot): prodissoconch bivalves (b), gastropods (c), copepods (d), ostracods (e), gammarids (f), fishes (g), caprellids (h).

individuals which fed largely (high P_i) on relatively infrequent (low % O) prey, such as isopods and prodissoconch bivalves.

Seasonal changes

The MDS plot obtained by pooling fish for each month of sampling is reported in Fig. 3. At an arbitrarily chosen similarity level of 60%, three main groups were identified, representing roughly a winter season (ws, from November to February), a spring-early summer season (ss, from March to July) and a late summer-autumn season (as, from August to October) (Fig. 3a). The stress value of the ordination was low (0.07), indicating a good representation of diet similarities among seasons in the two-dimensional scale (Clarke & Warwick 1994). According to the ANOSIM results, the diet of *B. affinis* is statistically different among the three periods compared (see global test, Table III). In particular, both ws and ss were well separated from as (R > 0.75), whereas ws and ss were overlapping but clearly different (R > 0.5) (Clarke & Gorley 2001). On the basis of the SIMPER analysis, we were able to identify the relative contribution of each prey

taxon to the ordination plot reported in Fig. 3a. Most of diet dissimilarity between ws/ss and as was largely due to prodissoconch bivalves, copepods and gastropods, whose pooled relative contribution to the average dissimilarities accounted for about 50-55 %. Prodissoconch bivalves were eaten exclusively during as (Fig. 3b), whereas copepods and gastropods were eaten almost exclusively during ws and ss (Fig. 3c, d). On the other hand, diet dissimilarity between ws and ss was mainly due to copepods, ostracods, gammarids, fishes and caprellids, which contributed together to 45 % of the average dissimilarities between the two groups. Copepods were preferably preyed upon during ws (Fig. 3d), whereas all other prey taxa mentioned above were consumed in large amounts during ss (Fig. 3e-h).

No significant difference in the fullness index (I_F) was found among seasons (H Kruskal-Wallis = 3.35, P > 0.05) (Table II). On average, I_F ranged between 0.50 in spring and 0.70 in winter. Levin's standardised index of diet breadth was highest in spring, with very low values in all other seasons (Table II).

Ontogenetic shift

The MDS plot obtained by pooling fish in 1 mm size classes is shown in Fig. 4. Excluding the smaller size classes (i.e. 13 and 14 mm) with only few specimens each, 23 fish size classes ranging from 15 mm to 37 mm were available for ontogenetic shift analysis. Assigning arbitrarily a threshold size of 23 mm L_t, small individuals (s, 15-23 mm L_t) and large individuals (l, 24-37 mm L_t) clustered together at a similarity level of 45 % (Fig. 4a). The stress value of the ordination plot was still low (0.12). Applying the SIMPER routine, the difference in diet of small-sized individuals (s) and large-sized individuals (l) was statistically significant (Table III), well separated from each other (R > 0.75) (Clarke & Gorley 2001). On the basis of the SIMPER analysis, most of diet dissimilarity between s and l was due to prodissoconch bivalves and copepods (Fig. 4b,c), yelding together about 44 % of

Table III. – One-way statistical analysis results (ANOSIM routine, test R). Groups are defined in the text.

Groups/Test	R statistic	Significance level
Global test		
all season (ws, ss, as)	0.77	0.001
Pairwise test		
ws, ss	0.69	0.008
ws, as	0.89	0.029
ss, as	0.89	0.018
Global test		
fish size (s, l)	0.82	0.001

Table II. – Ontogenetic shift and seasonal changes in the fullness index (I_F) (mean values and 95 % confidence intervals in brackets) and in diet breadth (Levin's standardised index B_A) (95% jackknife confidence intervals in brackets). L_t = total length; n = number of specimens.

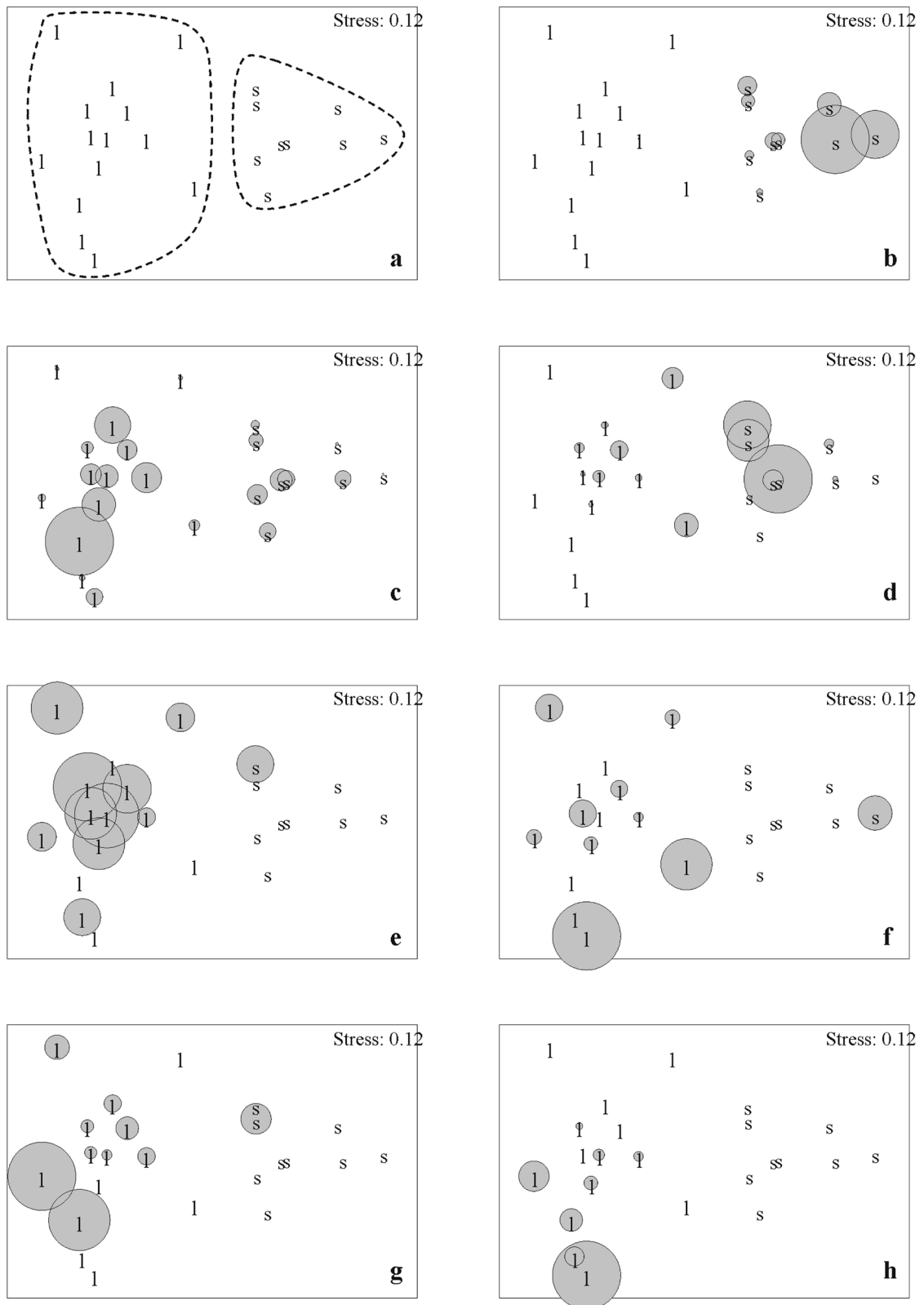| L_t (mm)	n	I_F	B_A	Season	n	I_F	B_A
16-23	61	0.69 (0.47-0.92)	0.047 (0.016-0.078)	spring	60	0.58 (0.33-0.83)	0.264 (0.095-0.433)
24-37	179	0.57 (0.43-0.72)	0.062 (0.034-0.091)	summer	60	0.64 (0.40-0.88)	0.033 (0.010-0.056)
				autumn	60	0.50 (0.32-0.67)	0.076 (0.037-0.116)
				winter	60	0.70 (0.11-0.79)	0.040 (0.009-0.070)

Fig. 4. – MDS analysis based on pair-wise similarity matrix derived from the mean numerical abundance of prey taxa in each fish size class. (a) MDS ordination plot of 1 mm fish size classes grouped in small individuals (*s*) and large individuals (*l*); bubble plots show mean numerical abundance pattern of the following prey taxa (superimposed to the relevant MDS plot): prodissoconch bivalvia (b), copepods (c), ostracods (d), gammarids (e), mysids (f), gastropods (g), fishes (h).

average dissimilarity between them. Prodissoconch bivalves were found exclusively in the stomach contents of small individuals (s) (Fig. 4b), whereas copepods were eaten preferentially by large individuals (l) (Fig. 4c). The cumulative contribution to the average dissimilarity of all other taxa was about 28 %, giving an overall value of 72 %. In detail, ostracods were the preferred prey of small individuals (s) (Fig. 4d), whereas gammarids, mysids, gastropods and fishes were consumed almost exclusively by large individuals (l) (Fig. 4 e-h).

Following the results of the ontogenetic shift in diet composition, the Mann-Whitney U-test was performed between small-sized individuals (16-23 mm L_t) and large-sized individuals (24-37 mm L_t). The Mann-Whitney U-test ($Z = -3.99$, $P > 0.001$) showed significant variation of the fullness index (I_F) between small individuals and large individuals. Levin's standardised index of diet breadth was low in both small and large individuals (Table II).

DISCUSSION

Despite gobiids being one of the largest groups of fish in inshore marine, estuarine and fresh water environments (Miller 1986), there is still little information on the biology of many species inhabiting the Mediterranean and north-eastern Atlantic. In particular, only one third of European marine gobiid species provide any published data on feeding habits (Kovačić 2001). The few data on diet of *B. affinis* available in the literature are those reported from Banyuls-sur-Mer (Zander & Berg 1984), if we consider valid the hypothesis of a misidentification of this species with *B. jeffreysii* on the basis of their distinct distribution (Kovačić 2002, Froese & Pauly 2007).

Present results indicate that *B. affinis* is carnivorous as are most of gobiids (Miller 1986). On the basis of the modified Costello plot (Amundsen *et al.* 1996), *B. affinis* can be considered a generalist feeder, relying generally on taxa with low prey-specific abundance (Pi). This conclusion is apparently in contrast with the low value of Levin's standardised index obtained for the whole population. However, it should be taken into account that Levin's index generally down-weights the rarer prey species which form only a minor component of the diet (Marshall & Elliott 1997). As a result, it is heavily influenced by the overwhelming numerical dominance in the diet of *B. affinis* of copepods and prodissoconch bivalves, which accounted for more than 80 % of all prey ingested, but present in the minority of the specimens. Compared to the modified Costello plot, the pooled data in Levin's standardised index lost the information on individual prey taxa kept in the modified Costello plot.

Despite its small size, *B. affinis* relied on a wide size range of prey, from copepods and prodissoconch bivalves to fish, larger crustaceans and polychaetes. However, the meiofauna dominated both numerically and in terms of frequency of occurrence, while the macrofauna dominated in terms of biomass. The same difference was noticed in the diet of other small epibenthic gobiids, such as *Gobius roulei* and *G. vittatus* (Kovačić 2001, 2007). This confirmed the importance to provide all three measures *(% N, % W, % O)* for describing fish diet, in order to provide a more exhaustive picture of food composition. A single index calculated from measures, like *Iri*, can loose information contained in the original measures (Berg 1979) and should be used only in addition to the data from the original measures.

Comparing present results with those provided by Zander & Berg (1984) from Banyuls-sur-Mer, huge differences in diet composition appeared between the two Mediterranean populations of *B. affinis*. The most frequent and numerically abundant prey differed completely between the two sites, despite the similarity of habitat between them i.e. sandy bottoms at 5-10 m depth (the Kvarner area) and 10-11 m (Banyuls-sur-Mer) (Zander & Berg 1984, unfortunately, the research didn't provide prey mass estimations). In particular, the population of *B. affinis* from the Kvarner area relied mainly on meiofauna, whereas the population from Banyuls-sur-Mer fed mainly on macrofauna, mainly consisting of amphipods, decapods and fish (Zander & Berg 1984). Different competition (and competitors) for food and prey availability between the two sites may explain the aforementioned difference in diet composition. However, the results from Banyuls-sur-Mer were from June, and the March to July samples from the Kvarner area were dissimilar in numerical abundance in the diet with other seasons in consuming in large amount macrofauna (gammarids, fishes and caprellids). The additional problem for comparison is small sample (n = 8) and large body size ($L_t > 36$ mm) of specimens from Banyuls-sur-Mer. This comparison showed low significance of research data limited by sample size, by season or by body size of studied specimens for conclusions on fish general feeding habits.

From an ecological perspective, in the Kvarner area *B. affinis* fed on several prey taxa that live on the bottom (i.e. epibenthic) or close to it (i.e. hyperbenthic). Organisms belonging to deeper infauna were not present in the diet, or they were very rare. Combined diet composition of benthic and hyperbenthic prey was rather common in other epibenthic gobiid species living inshore on sandy bottoms, such as *Deltentosteus quadrimaculatus*, *Gobius geniporus, G. roulei, Pomatoschistus bathi*, *P. minutus* and *P. pictus* (Kovačić 2001, Villiers 1980, Zander 1982, Zander & Hagemann 1987, 1989, Zander & Heymer 1992). Nevertheless, the relative contribution of meiofauna and macrofauna to the species diet composition depended mostly on the predator size. On the other hand, hyperbenthic juveniles of *D. quadrimaculatus, P. minutus*, and *P. pictus*, as well as the hyperbenthic *Pomatoschistus quagga* fed mostly on plankton (Villiers 1980,

Zander 1982, Zander & Hagemann 1987).

The diet composition of *B. affinis* in the Kvarner area was significantly different between small- and large-sized individuals, switching from meiofaunal to meio-macro-faunal prey as they approach approximately 23 mm *Lt*. A similar ontogenetic shift in diet in the same area and habitat was observed also in larger *G. roulei*, that switched from meio-macrofaunal to macrofaunal prey (Kovačić 2001). Therefore, other than prey availability in the local environment, some morphological constraint like the mouth opening and dental characteristics probably play a key role in determining the food composition in these gobiid fishes.

In contrast to previous results provided for other gobiids (Joyeux *et al*. 1991, Kovačić 2001, 2007), no seasonality was observed in the feeding intensity of *B. affinis*. Conversely, diet composition was significantly different among seasons, as evidenced in other Mediterranean gobiids (Kovačić 2001, 2007, Zander & Hagemann 1989). To some degree, it could be related to a coupled effect of different seasonal prey availability and fish size, as most juveniles *B. affinis* were collected in summer. The occurrence in the stomach contents of specimens sampled in August and September of prodissoconch bivalves, which represents a development stage just before and at the settlement of larvae, matched well with summer spawning of many bivalves in the area (M Hrs-Brenko pers comm). Thus, the seasonal preferences in the diet composition of small epibenthic littoral fishes could be the result of seasonal occurrence of suitable prey (Zander & Hagemann 1989).

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