

Anthropogenic effects on reproductive effort and allocation of energy reserves in the Mediterranean octocoral *Paramuricea clavata*

Georgios Tsounis^{1,3,*}, Lucia Martinez¹, Lorenzo Bramanti¹, Nuria Viladrich², Josep-Maria Gili¹, Ángela Martínez¹, Sergio Rossi²

¹Institut de Ciències del Mar (CSIC), Passeig Marítim de la Barceloneta, 37–49, Barcelona 08003, Spain

²Institut de Ciència i Tecnologia Ambientals (Universitat Autònoma de Barcelona), Edifici Cn Campus UAB, Cerdanyola del Vallés, Barcelona, 08193, Spain

³Present address: Leibniz Center for Tropical Marine Ecology (ZMT), Fahrenheitstrasse 6, 28359 Bremen, Germany

ABSTRACT: In order to better understand the sources, patterns and consequences of anthropogenic effects on populations of the Mediterranean gorgonian *Paramuricea clavata*, we examined the proportion of injured colonies among populations exposed to a combination of anthropogenic disturbances (recreational cast fishing, commercial lobster pots, gill nets and SCUBA diving), as well as the physiological response of injured corals. Between 10 and 33% of the colonies in unprotected populations were partially colonized by epibionts, most likely following tissue injury, whereas only 4 to 10% of the populations in a marine protected area were affected. Populations that were simultaneously exposed to fishing as well as intensive SCUBA diving showed the highest proportion of colonization. Colonies with approximately 30 to 35% of epibiont coverage showed significantly lower numbers of gonads per polyp. Similarly, concentrations of lipids were lower in females with epibionts, thus indicating allocation of resources into recovery of injured tissue instead of reproduction. Furthermore, whereas unaffected colonies showed a uniform distribution of carbohydrates and proteins through apical branches to more central ones, colonies with epibionts had significantly lower protein concentrations in branches that are positioned 3 branching order levels closer to the stem. The results thus indicate a preference of apical growth in recovering colonies, via a different distribution of food within the colony. Reproductive success in surface-brooding corals growing on walls and overhangs might also be reduced by SCUBA bubbles from divers passing below, as bubbles efficiently remove eggs brooded on colony branches.

KEY WORDS: Fecundity · Bubbles · Fishing · Injury · Epibiosis · SCUBA · Gorgonian · Protein-carbohydrate–lipid balance · Human impact

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INTRODUCTION

Understanding how coral communities respond to anthropogenic impacts is becoming ever more important in light of the increasing use of coastal areas (Jackson et al. 2001, Knowlton & Jackson 2008). Mediterranean coralligenous habitats are arguably Europe's most diverse marine habitats, in part be-

cause of benthic key species that provide 3-dimensional structure (Ballesteros 2006), such as gorgonian corals.

Recent studies have revealed a threat to the survival of some populations of the Mediterranean gorgonian *Paramuricea clavata* as a result of recreational SCUBA diving and climate-related mass mortality events (Coma et al. 2004, Linares & Doak

*Email: georgios.tsounis@zmt-bremen.de

2010). Although the impact of climate anomalies on this species has been the focus of several recent studies (e.g. Perez et al. 2003, Linares et al. 2005, Cupido et al. 2008), other sources of mortality in *P. clavata* have received less attention, such as mortality due to tissue damage by fishing lines (Bavestrello et al. 1997, Harmelin & Marinopoulos 1994) or suffocation by coverage of mucilage produced by filamentous algae (Mistri & Cecherelli 1993).

Among these factors, impact by SCUBA diving and commercial or recreational fishing stand out because of their more widespread and constant nature, as well as their increasing intensity in recent decades (Diedrich 2007). Furthermore, both activities concentrate on sites of high biodiversity and productivity, which often coincide with rich octocoral communities, and both activities inflict tissue damage to benthic organisms through direct physical contact with divers (Zakai & Chadwick-Fuhrman 2002, Garrabou et al. 1998, Sala et al. 1996) or fishing gear, respectively (Yoshikawa & Asoh 2004).

Because of the flexibility of their gorgonin skeleton, horny corals are not prone to breakage of branches (Lin & Dai 1996). Instead, studies indicate high mortality rates through death by detachment of whole colonies in areas of high diving intensity or wave impact (Yoshioka & Yoshioka 1991, Coma et al. 2004). Yet it can be postulated that contact with light gear and divers (rather than dredges and anchors) results more often in tissue wounds of various degree than in colony detachment.

In any case, the frequency of small injuries may be masked by rapid regeneration (Lindsay 2010), as lesions can either be colonized by epibionts and become permanently evident, or they can heal and go unnoticed by re-covering the denuded skeleton axis with living tissue (Wahle 1983, Castanaro & Lasker 2003, Linares et al. 2005, Cupido 2009). Abrasion is, after all, a natural phenomenon as it occurs as a consequence of gastropod predators (Theodor 1967) or wave action (Grigg 1976). Soft corals have therefore developed recovery mechanisms (Sánchez & Lasker 2004), and are able to quickly heal minor and momentary injuries by growth of a new coenenchyme that prevents the settlement of pioneer epibiont species such as hydroids (Loya 1976). Extensive or repeated injuries, in contrast, are eventually colonized by bryozoans, which cannot be removed and begin a permanent colonization phase of a varied epibiont community (Riegl & Riegl 1996, Bavestrello et al. 1997, Cerrano et al. 2005). Furthermore, regeneration rates are inversely correlated to the extent of

injuries (Cupido 2009); consequently, recovery from frequent or extensive damage may be difficult and thus lead to the death and subsequent detachment of the colony.

As tissue regeneration is constantly counterbalancing injury, it is a critical process that needs to be thoroughly understood to make predictions about the survival of stressed gorgonian populations. Specifically, as tissue regeneration comes at an energetic cost, it affects the balance and quantity of energy reserves, thus resources normally used for reproduction may be allocated to regeneration (Silveira & Van't Hoff 1977, Rinkevich 1996, Henry & Hart 2005). This is in fact consistent with a recently observed reduction of fecundity in a population recovering from mass mortality (Linares et al. 2008a). However, unexpected rates of tissue regeneration and growth of new branches have been documented in *Paramuricea clavata* (Cupido 2009), raising the question whether an overcompensation response exists as it does in some tropical gorgonian corals (Sánchez & Lasker 2004).

In order to improve our understanding, the first objective of this study was to quantify the proportion of injured colonies in *Paramuricea clavata* populations in the peninsula of Cap de Creus and the Medes Islands on the Costa Brava (Spain), while comparing areas that are exposed to different types of human impact, i.e. recreational or commercial fishing, SCUBA diving or combinations of these impacts, with an undisturbed reference area to identify injury causes.

The second objective was to study how injured colonies respond in terms of polyp fecundity and level of macromolecular energy reserves. The suitability of macromolecules as an index of nutrition and health state of *Paramuricea clavata* has been shown in previous studies (Rossi et al. 2006a, Gori et al. 2007, Rossi & Tsounis 2007, Tsounis et al. 2007), and is based on the principle that octocorals under favorable conditions store energy reserves in form of proteins and especially lipids (Stimson 1987, Brockington et al. 2001).

This study hypothesized that injury to gorgonians initiates a tissue regeneration response, and thus has implications for energy reserve allocation and sexual reproduction. Furthermore, as gorgonian corals normally transport food particles to branches that are less efficient in capturing food (Murdock 1978a,b), it was also hypothesized that injured colonies should show a different distribution of macromolecular energy reserves, revealing a change of food transport patterns as a response to injury.

The third objective of this study was to address the impact of SCUBA bubbles on externally fertilized surface-brooding corals (see Benayahu & Loya 1983). In *Paramuricea clavata*, fertilization is external, i.e. the eggs are brooded in mucus on the branch surface until, when fertilized, they transform into larvae and are finally swept away by currents when the mucus fails to hold them (Coma et al. 1995, Linares et al. 2007, Linares et al. 2008b, G.T. unpubl. data). We measured the effect of bubbles and postulate that they may reduce fertilization success of gorgonians in their typical habitat niche (arches, walls and overhangs) by removing unfertilized eggs from the branches of female colonies before nearby males release their sperm to fertilize them. Although not on the same scale as injuries from divers and fishing gear, we believe this aspect deserves consideration within management plans.

MATERIALS AND METHODS

Study area

The study was carried out at the Cap de Creus peninsula and the Medes Islands (Fig. 1), which are both located on Spain's northern Mediterranean coast within the Gulf de Lion. Cap de Creus consists of >3000 ha and 42 km of coastline and was declared a marine park in 1996. However, only 1 site (Stn 7 in Fig. 1a, Illa Encalladora) is a marine protected area (MPA), where fishing and diving are prohibited. The rest of the peninsula is used by recreational and artisanal fishers, SCUBA tour operators and spear fishermen (Gómez i Mestres 2003, Lloret 2003a,b, Lloret & Riera 2008). Although fishing is managed in some areas and prohibited in others, the number of divers is not managed in Cap de Creus, and anchoring occurs at a few sites. In contrast, the nearby Medes Islands (Fig. 1) comprise a coastal marine park where fishing and anchoring are prohibited and SCUBA diving is managed (Table 1). However, the limitation of the number of divers permitted each year appears to exceed typical recommendations for other sites (Zakai & Chadwick-Fuhrman 2002, Coma et al. 2004, Linares & Doak 2010).

The study area is characterized by a coralligenous community that rivals coral reefs in structural complexity (Ballesteros 2006). Populations of the gorgonian *Paramuricea clavata* (Risso, 1826) are a common and characteristic component of these communities, and are often found in hard-bottom habitats (typically walls and overhangs) exposed to swift currents

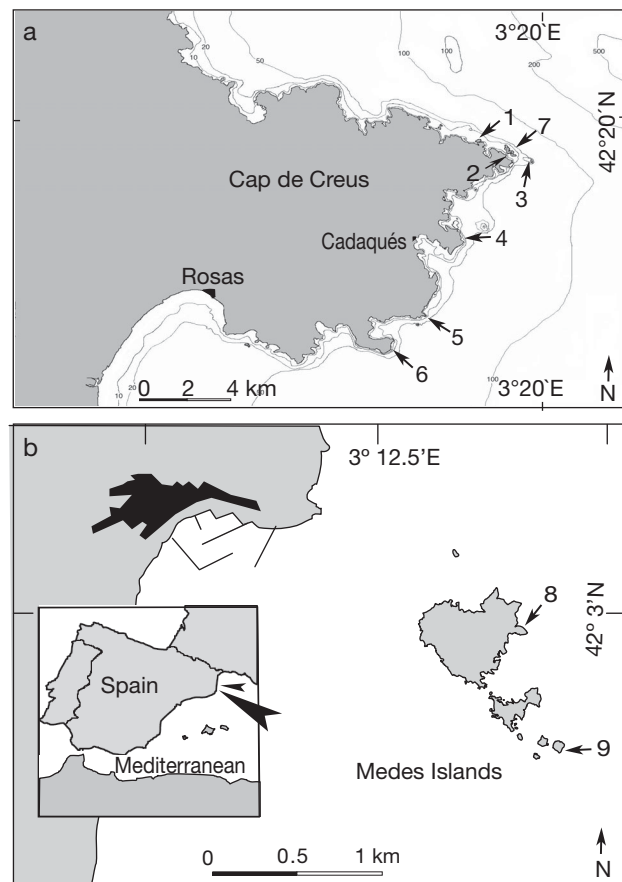


Fig. 1. Study areas: Cap de Creus (a) and Medes Islands (b). Numbers mark the sampling stations: 1, Illa Culleró; 2, Es Freu de Clavaguera; 3, Illa Massa d'Or; 4, Punta de S'Oliguera; 5, Punta de sa Figuera; 6, Cap Norfeu; 7, Illa de la Encalladora; 8, Cova de la Vaca; and 9, Carall Bernat

at 16 m depth and below. *P. clavata* is a slow growing, long-lived, dioecious, surface-brooding octocoral with external fertilization and locally synchronized annual spawning in summer (Coma et al. 1995), i.e. female colonies brood planula larvae on their branches and liberate them on several distinct events correlated to new and full moon phases in June.

Population state

Surveys by SCUBA divers along transect lines were used to determine the proportion of colonies with injuries and/or epibionts over a wide area in several populations. Populations were defined as distinct patches or groups of colonies, but not as geneti-

Table 1. *Paramuricea clavata*. Injuries and epibionts in populations at the Cap de Creus peninsula and Medes Islands MPA. Effect types: recreational cast fishing (RF); SCUBA diving (SC); anchoring (A); gill nets (GN); lobster pots (LP); sedimenting shell debris (SB); and spear fishing (SF). Bold numbers = absolute number of incidents observed, numbers in brackets = respective percentage values. * = illegal fishing. *Parerythropodium coralloides* was not included in the epibiont count, except in the column 'total epibiosis'. Stns 1, 4a and 7 were further sampled for energy balance and fertility analysis

Date	Station/Site	Impact type	Depth (m)	Nylon lines	Pale branches	Recent injuries	Dead colonies	<i>P. coral-loides</i>	Other epibionts	% total epibiosis	No. colonies	Transect area (m ²)	Abundance (ind. m ⁻²)
Cap de Creus													
24.6.10	1 - Illa del Culleró	RF, LP	20	11 (5)	5 (2)	10 (4)	7 (3)	0	22 (10)	10	231	34	7
25.6.10	2 - Es Freu de Clavaguera	SB	16	1 (0.4)	6 (2)	9 (3)	23 (9)	11 (4)	33 (13)	17	259	22	12
23.6.10	3a - Illa de Maça d'Or	RF, SC, A	17	0	7 (2)	20 (5)	12 (3)	1 (0.0)	35 (9)	10	372	27	14
19.7.10	3b - Illa de Maça d'Or	RF, SC	27	0	6 (4)	5 (3)	2 (1)	0	40 (24)	24	166	12	14
24.6.10	3c - Illa de Maça d'Or	RF, SC, A	30	4 (6)	8 (11)	1 (1)	0	2 (3)	7 (10)	13	70	4	18
24.5.10	4a - Punta de S'Oliguera	RF	19	2 (1)	24 (10)	3 (1)	7 (3)	3 (1)	53 (22)	23	240	48	5
24.5.10	4b - Punta de S'Oliguera	RF	20	2 (1.1)	3 (1.7)	1 (6)	1 (6)	0	2 (1.1)	11	18	4	5
10.7.10	5 - Punta de sa Figuera	SF	16	1 (0.5)	11 (5)	2 (1)	6 (3)	10 (5)	32 (15)	19	220	32	7
23.6.10	6a - Cap Norfeu	SC	19	0	10 (3)	2 (1)	5 (2)	17 (6)	48 (16)	22	294	40	7
25.6.10	6b - Cap Norfeu	SC	27	1 (0.3)	4 (1)	5 (2)	14 (4)	14 (4)	19 (6)	10	329	22	15
20.7.10	6c - Cap Norfeu	SC	38	2 (2)	2 (2)	2 (2)	3 (3)	11 (12)	19 (21)	33	91	10	9
14.01.11	7 - Illa Encalladora MPA	-	30	0	3 (1)	6 (2)	3 (1)	5 (2)	15 (5)	7	290	40	7
14.01.11	7 - Illa Encalladora MPA	-	20	1 (0.0)	2 (1)	2 (1)	2 (1)	7 (4)	10 (6)	10	170	18	9
15.01.11	7 - Illa Encalladora MPA	(RF, LP)*	22	1 (0.0)	5 (1)	22 (6)	10 (3)	2 (1)	13 (4)	4	347	30	12
15.01.11	7 - Illa Encalladora MPA	(RF, LP)*	18	1 (0.0)	2 (1)	10 (7)	1 (1)	1 (1)	6 (4)	5	151	10	15
Medes Islands													
25.8.10	8 - Cova de la Vaca	SC	19	0	2 (1)	0	8 (4)	22 (11)	37 (18)	29	201	20	10
25.8.10	9 - Carall Bernat	SC	25	0	6 (2)	7 (3)	17 (7)	3 (1)	22 (9)	10	255	20	13

cally isolated groups. Between June 2010 and January 2011, 15 surveys at 9 locations at Cap de Creus and the Medes Islands were carried out by SCUBA divers along a transect line on isobaths between 16 and 38 m depth, examining a 2 m wide zone over a transect length of 6 to 20 m, depending on whether the size of the population permitted the full length (Table 1). The chosen sites differed in their exposure to anthropogenic factors, as some areas are popular SCUBA diving or recreational cast fishing sites (accessed either from land or by boat), and other areas are fished commercially using gill nets and lobster pots (Table 1). Some populations were only exposed to SCUBA diving, and 1 is completely protected and served as a baseline reference (Illa Encalladora). The sites are very similar in environmental features and are thus comparable (see also Tsounis et al. 2007). Given that the study area is not known to have suffered from mass mortality events as detected at the French and Italian coast (Cupido et al. 2009), the surveys were designed to determine the effects of direct human impact. Furthermore, as wave action and mass mortality events do not appear to affect populations at approximately 35 to 40 m depth (Cupido 2009), transects at different depths were employed to distinguish human impact.

The same divers recorded the following parameters throughout the study by direct observation: presence of monofilament fishing lines among the gorgonian branches; presence of recovering tissue (pale color); recent injury (denuded skeleton axis with no epibiosis); epibiosis by *Parerythropodium coralloides*; other epibionts (mainly bryozoans); and completely dead colonies. Colonies were defined as affected by epibionts regardless of how small the epibiont coverage, and comparisons focused on the proportion of colonies with epibionts and those without. This approach allowed us to determine the uninjured proportion of the populations quickly to characterize a wider area. A chi-square test was used to test for sig-

nificant deviation of unprotected populations from the expected ratio between the number of colonies with epibionts and the unaffected colonies. The expected baseline value of no disturbance was defined by the ratio determined along the transects within the protected area.

Fecundity and balance of energy reserves

The experimental design for testing the hypothesis that colonies with epibiosis allocate energy reserves into tissue growth consisted of separate tests for differences in gonad number per polyp, sum of gonadal volume per polyp, as well as tissue concentration of lipids, proteins and carbohydrates.

In order to test for the hypothesis that injuries change the distribution pattern of energy storage macromolecules among different parts of the colony, we compared the concentration of proteins and carbohydrates in the first 3 branch orders in 6 colonies affected by epibiosis with the concentration of 6 unaffected ones. Branching pattern was characterized following Brazeau & Lasker (1988), where the apical branches are defined as first order, and branches towards the stem are of higher order, when 2 lower-order branches meet (i.e. 2 or more first-order branches grow from a second-order branch).

Sampling was carried out on 5 and 6 June 2010, within the crucial period when gonads are well developed but have not yet been released (for a baseline see Rossi et al. 2006a). This ensures that the observed energy reserve levels are the net result of nutrition, growth, recovery and reproductive investment over a period of many preceding months. As shallow water populations are small, we sampled 20 unaffected and 20 affected colonies from each of 3 distinct replicate populations (Illa Cullero, Punta de sa Oliguera and Cova de la Vaca). The sampled colonies were located at 19 m depth and measured ca. 40 cm in colony height. The tips of the first-order branches were used for biochemical analysis, whereas the basal part of the first-order branch was used to identify the sex of the colony and determine its fecundity (see below). In the case of the branching order comparison, larger samples were cut to include 3 branching orders. Colonies were defined as unaffected when they showed no visible sign of epibiosis, whereas the affected colonies chosen for sampling were colonies that had approximately 30–35% of their branches covered by epibionts. Because the objective was to study

how epibiosis of injured branches affects the whole colony, the sampled branches were unaffected branches that were located on the unaffected side of the colony, but adjacent to its overgrown part.

Samples for biochemical analysis were frozen in liquid nitrogen within 30 min of collection and transported to the laboratory, where they were kept at -80°C until they were freeze-dried for 12 h at -110°C and a pressure of 100 mbar, and finally stored frozen at -20°C , pending analysis.

Organic matter content (or ash-free dry mass, AFDM) of first-order branches was calculated by heating 10–20 mg of tissue (10–20 polyps per branch and connective tissue) of each sample at 80°C for 48 h and weighing the sample to obtain dry mass. The tissue was then heated for 5 h at 450°C , and weighed again. The difference between dry mass and ash mass expresses the organic matter (Rossi et al. 2006a), which was used for normalization. All the protein–carbohydrate–lipid analyses were performed spectrophotometrically (colorimetrically).

Carbohydrate analysis used 7 mg of tissue (without using the skeletal axis). The tissue was weighed using a microbalance (precision: ± 0.01 mg), and was homogenized in 3 ml of distilled water. Carbohydrate concentrations were determined applying the method of Dubois et al. (1956), using glucose as standard. For protein analysis, 5 mg of tissue was homogenized in 3 ml of distilled water and 1 ml 1 N NaOH to quantify protein concentration applying the method of Lowry et al. (1951), using bovine serum albumin as standard. Analysis of lipids required 10 mg dry tissue, which was homogenized in 3 ml of chloroform-methanol (2:1 v/v). Lipid concentrations were quantified using the method of Barnes & Blackstock (1973), with cholesterol as standard. These methodologies have been previously applied to other anthozoans (Ward 1995, Rossi et al. 2006b, Rossi & Tsounis 2007, Gori et al. 2007), as well as *Paramuricea clavata* (Rossi et al. 2006a); therefore, more modern protocols were avoided to be able to compare results.

Comparisons were carried out separately for both sexes as lipid levels in *Paramuricea clavata* differ between males and females in spring (Rossi et al. 2006a). However, when comparing branch order effects, the small sample size required pooling the data, so lipid levels could not be compared because of the known differences between sexes. Data were analyzed using a univariate 3-way PERMANOVA with the factors location (3 levels), sex (2 levels, males and females) and epibiosis (2 levels, unaf-

ected and affected by epibionts). Homogeneity of variances was tested using the program PERMDISP, and a pairwise test was applied for post hoc testing (Anderson & ter Braak 2003).

The basal (closer to the stem) part of the samples described above was used to compare the fecundity in colonies with epibionts and unaffected colonies. The samples were fixed in 6% formaldehyde until analysis. A total of 5 polyps on each branch were dissected and the sperm sacs or ovaries were counted and measured using a binocular microscope (Coma et al. 1995). In order to reduce the variability in gonadal volumes, the data were pooled into total gonadal volume per sampled colony (5 polyps).

The data were found to be heteroscedastic and deviated from a normal distribution. Transformation could not rectify this; therefore, PERMANOVA analyses were used to compare differences between colonies with epibiosis and unaffected colonies in either number of gonads per polyp, or sum of gonadal volume per 5 polyps, for each sex and sampling station. No transformation or standardization was conducted on the raw data and the analysis used Euclidean distances (9999 permutations). The PERMANOVA enabled a conventional univariate ANOVA approach to be conducted using the concepts and assumptions of a multivariate method and included the use of pairwise comparisons (Anderson & ter Braak 2003).

Effect of SCUBA bubbles on brood care

The quantity of exhaled SCUBA air bubbles that is able to considerably reduce the number of eggs being held by mucus to the branches of *Paramuricea clavata* during the brooding phase was measured *in situ*. A known quantity of simulated SCUBA bubbles was created by filling an inverted 2 l measuring cylinder with air and releasing its content below one colony in small portions (ca. 0.5 l). This is roughly equivalent to the typical respiratory volume of a diver during low work load (Bove & Davis 1997). The colony was filmed during the experiment, so that the number of eggs could be roughly estimated before and after exposure to bubbles. The experiment was not replicated, as variations in mucus strength, bubble velocity or stream focus were judged insignificant in comparison to real-world impact. Due to large numbers of divers at popular dive sites, real-world effects are exponentially more efficient in removing eggs than the few simulated breaths.

RESULTS

Population state

Between 10 and 33% of the colonies in the unprotected populations were affected by epibionts (Table 1), whereas protected populations differed significantly, as only 4 to 10% of the colonies were affected ($\chi^2 = 15.6$, $p = 0.0004$, $df = 2$). No depth gradient in the proportion of colonies with epibionts was found between 4 and 38 m depth. Some of the deeper populations even showed a proportion of colonies with epibionts that was as high as in the most affected shallow populations: Maca d'Or at 30 m (13%) and Cap Norfeu at 38 m (33%). High spatial variability was evident, as the transect with the lowest epibiosis was close to one of the highest in terms of proportion of colonies with epibionts: Cap Norfeu at 27 m (10%) and Cap Norfeu at 38 m (33%).

The epibionts observed on the colonies were hydroids, bryozoans, filamentous and calcareous algae, and the parasitic gorgonian *Parerythropodium coralloides*. Although the former groups were present in all stations, the presence of *P. coralloides* varied extremely, being absent or rare at half of the stations. The highest proportion of corals colonized by this parasitic octocoral were found at the stations that are known to be favorite SCUBA diving sites, receiving tens of thousands of visitors each year: 11% at Cova de la Vaca at the Medes Islands marine park, and 12% at Cap Norfeu at 38 m depth.

Monofilament fishing lines were most frequent at certain sites that are easily accessible to, or very popular among, recreational cast fishermen (Table 1). These were Illa Cullero (5% of the colonies were entangled in fishing lines) and Punta de sa Oliguera (11%), which are accessible by land, and Maca d'Or (6%), which is accessible by boat. Branches with pale tissue were observed, in some cases next to where fishing lines were in contact with the tissue (see Fig. S7 in Supplement 1 at www.int-res.com/articles/suppl/m449p161_supp.pdf).

Recent injuries were recorded in shallow-water populations at the stations Illa Culleró (4%), Maca d'Or at 16 m (5%) and Punta de sa Oliguera at 20 m (6%). At the latter, we also recorded the highest proportion of pale, smoother branches with smaller polyps (6%). The highest proportion of fresh injuries was detected in one of the populations situated in the strictly protected area (Illa Encalladora); these injuries were a result of an illegal fishing event.

Fecundity and balance of energy reserves

The concentration of lipids in apical branches was significantly higher in unaffected female colonies (Fig. 2) than in affected female colonies (i.e. colonies that are colonized by epibionts). No such difference was found in male colonies (significant interaction between the factors epibiosis and sex, $p = 0.01$, $F_{2,65} = 6.583$, $N = 78$). Carbohydrate concentration was significantly higher in unaffected than in affected colonies of both sexes (univariate 3-way PERMANOVA: $N = 78$, $F_{2,65} = 12.39$, $p = 0.0003$). Protein levels were significantly higher in unaffected male

colonies than in affected male ones ($N = 78$, $F_{2,65} = 74.49$, $p = 0.007$), whereas female colonies showed no difference.

The comparison of protein concentrations between first-, second- and third-order branches showed similar levels among branch orders of unaffected colonies, whereas in affected colonies there were significantly lower concentrations in higher branch orders, i.e. branches closer to the stem ($N = 9$, $F_{2,65} = 14.170$, $p = 0.0007$). In carbohydrates, the data show the same trend but the differences were not significant (Fig. 3).

Affected and unaffected colonies were fertile at all sites. However, the mean number of gonads per polyp was significantly lower in affected male and female colonies than in unaffected ones ($N = 78$, $F_{2,64} = 10.73$, $p = 0.001$; Fig. 4).

The comparison of the sum of gonadal volumes per sampled colony showed an effect of the factors epibiosis and sex (Fig. 5). Unaffected colonies showed significantly higher gonadal volumes than affected ones ($F_{2,64} = 7.1504$, $p = 0.009$, $N = 65$), and male colonies showed a higher gonadal volume than female ones ($F_{2,64} = 11.295$, $p = 0.001$, $N = 65$).

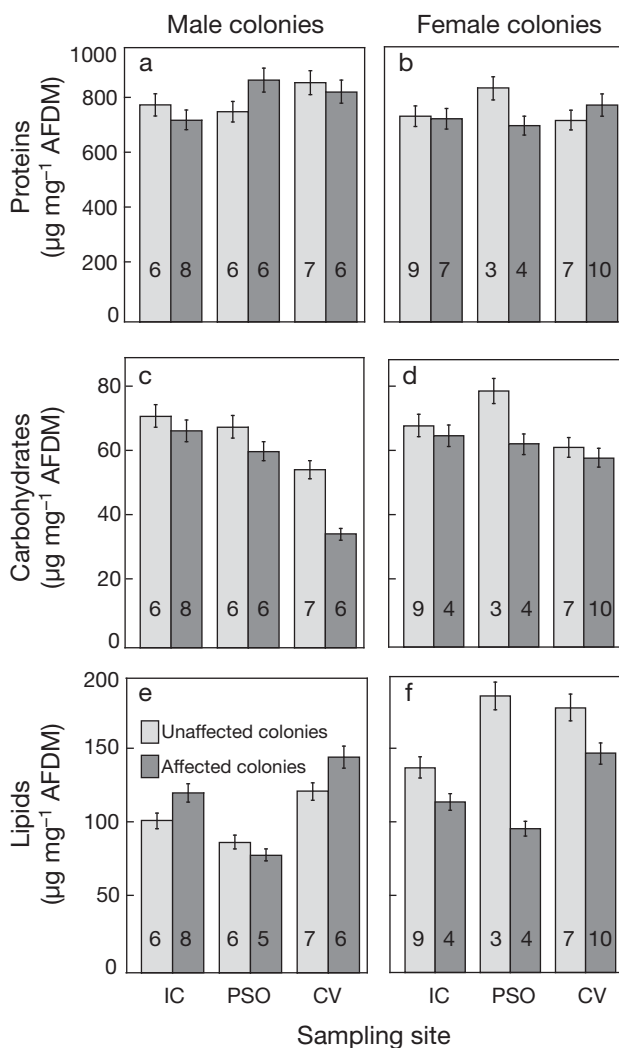


Fig. 2. *Paramuricea clavata*. Concentration of (a,b) proteins, (c,d) carbohydrates and (e,f) lipids (means \pm SD); $\mu\text{g mg}^{-1}$ AFDM (ash-free dry mass) in the tissue of unaffected colonies (light bars) and colonies affected by epibiosis (dark bars) at Illa del Culleró (IC), Punta de S'Oliguera (PSO) and Cova de la Vaca (CV). a,c,e: male colonies; b,d,f: female colonies. Numbers within columns = numbers of colonies sampled

Effect of SCUBA bubbles on brood care

The colony used for demonstrating the effect of SCUBA bubbles on brood care had 5 groups of mucus filaments containing eggs adhering to its branches. Observation of this and other colonies showed that even strong currents did not completely remove the eggs from the colonies over 48 h. However, <3 l of air bubbles released ca. 20 cm below the branches instantly detached >90% of the larvae from the colony (Fig. 6, see also the video in Supplement 2 at www.int-res.com/articles/suppl/m449p161_supp/).

DISCUSSION

Population state

The proportion of colonies that are affected by epibionts can serve as an indicator for the intensity of disturbance and health state, as injuries promote epibiosis (Bavestrello et al. 1997). For the sake of evaluating a larger area in the time available, we only distinguished between unaffected colonies and affected colonies with any visible degree of epibiosis, but did not distinguish between colonies with various degrees of epibiotic cover (but do suggest this focus

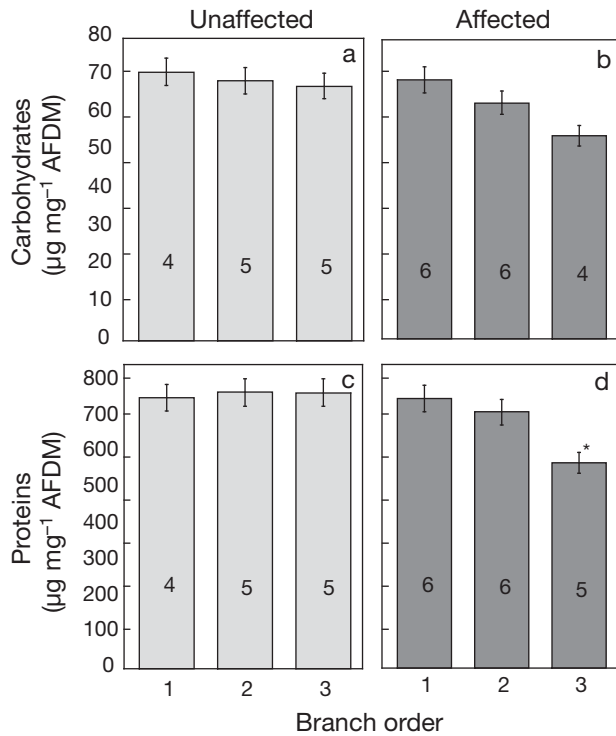


Fig. 3. *Paramuricea clavata*. Concentration of (a,b) proteins and (c,d) carbohydrates (means ± SD; µg mg⁻¹ AFDM [ash-free dry mass]) in the tissue of unaffected colonies (a,c) and colonies affected with epibiosis (b,d) in branches of different branching order. Numbers within columns = number of colonies sampled. *: significant difference

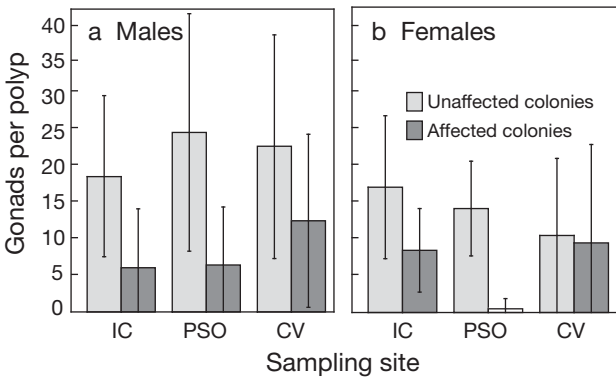


Fig. 4. *Paramuricea clavata*. Number of gonads per polyp (a) in males and (b) females at the 3 sampling sites (mean ± SD; total sample size: n = 65). Site codes see legend of Fig. 2

for future research). Despite this simplification, we found that unprotected populations contained a significantly higher proportion of colonies with epibionts. Descriptions of human usage of the area (Lloret 2003a,b, Gómez i Mestres 2003) and observations during the surveys allow classification into different types and intensity categories of usage (Table 1). This reveals that the highest epibiont rates

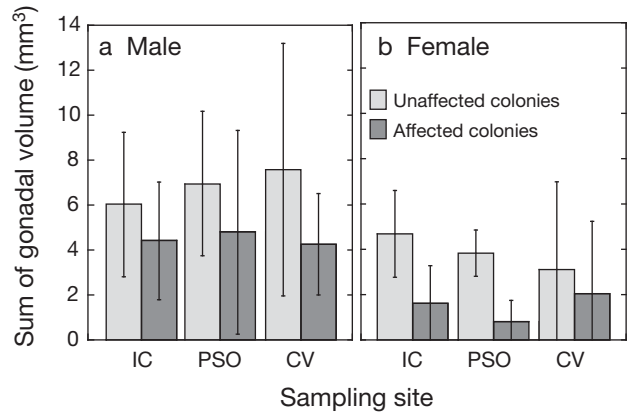


Fig. 5. *Paramuricea clavata*. Sum of gonadal volume within 5 sampled polyps per colony in males (a) and females (b) at the 3 sampling sites. Means + SD. Site codes see legend of Fig. 2

(24% and 33%) were found in populations that are exposed to a high intensity of SCUBA diving (Cap Norfeu), a combination of fishing and SCUBA (e.g. in Punta de sa Oliguera), or anchoring (at the islet of Maca d'Or). The 2 stations showing the highest colonization rate with the parasitic octocoral *Parerythroplodium coralloides* were 2 stations with intensive diving activity and no direct fishing impact (Cap Norfeu and Cova de la Vaca). These results indicate that injuries by divers may be especially frequent during the reproductive phase of this parasitic anthozoan.

Small-scale spatial variability in the proportion of affected colonies is most likely due to highly spatial patterns of human behavior: cast fishing from land concentrates on accessible areas, SCUBA divers follow scenic routes that are determined by practical SCUBA necessities, and anchoring spots are dictated by typical weather and topographic conditions. Surprisingly, areas that prohibit fishing were among the ones with the highest impact, perhaps because protection from fishing simply substitutes diving for fishing, and sites with protected fish life attract divers in high numbers.

As expected, the strictly protected area showed a significantly lower proportion of colonies that were affected by epibionts. However, even the MPA showed signs of (illegal) fishing, which indicates that enforcement of the MPA is not adequate (Table 1). The remains of animal bones that are usually used as bait indicate illegally deployed lobster pots, which caused tissue abrasion with their connecting ropes when retrieving the traps (see Figs. S3 & S4 in Supplement 1). As such, poaching makes it difficult to distinguish human from natural impact and establish a baseline of injury frequency under natural conditions. Future research could provide a more robust

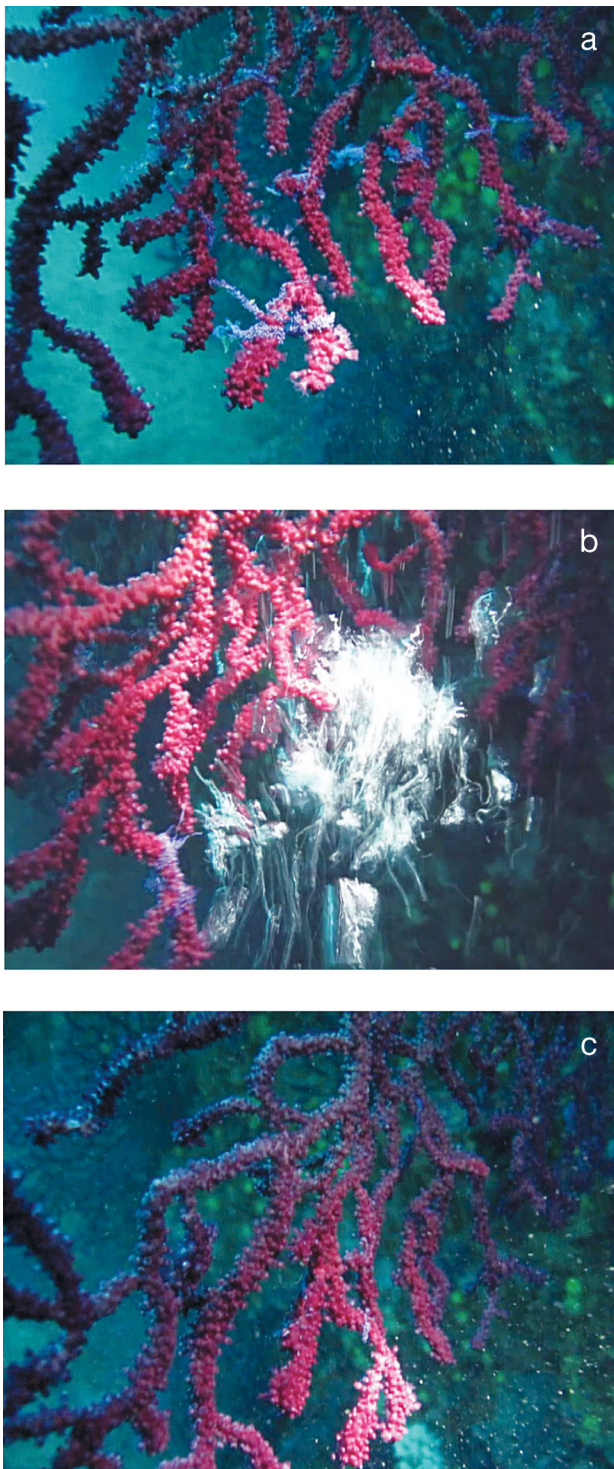


Fig. 6. Frame grabs from video footage documenting the experiment of the effect of SCUBA bubbles. (a) *Paramuricea clavata* colony with eggs being brooded on its branches prior to release of bubbles. (b) Bubbles washing through the branches. (c) Gorgonian branches with hardly any eggs left on its surface. Please see the video clip in Supplement 2 at www.int-res.com/articles/suppl/m449p161_supp/

data set for the natural baseline by sampling a broader and well-enforced area over a longer time frame and consider degrees of epibiont coverage. Both will allow stronger statistical comparisons than possible in the present study, so future research may document even higher human impact.

Some other observations help distinguish the simultaneous effects of various impact types, especially wave action and climate-related mass mortality events. Mass mortality events induced by climate anomalies have received increased attention recently, yet none have been recorded in our study area (Cupido 2009), and these events, where they do occur, do not appear to affect populations at 35–40 m depth (Garrabou et al. 2001, Bramanti et al. 2005, Linares et al. 2005, Cupido et al. 2008, Gori et al. 2010). Cap de Creus is known to receive alpine Tramontana winds that cause heavy winter storms which affect growth form (Velimirov 1976) and likely increase injury and mortality rates of gorgonians. As wave action should be less violent below 35 m, we expected an inverse gradient of epibiosis proportion with depth which, however, we could not confirm. Instead, we found locally high colonization rates, e.g. where the base of a wall is subjected to lost nylon lines or divers (Table 1).

The surveys also recorded an exceptional situation at Es Freu at 16 m depth, where a population is under locally high natural impact, which resulted in 17 % of the colonies being affected by epibionts. This population lives in a shallow canal with strong currents and large amounts of shell debris raining down on the gorgonians, thus causing suffocation and tissue damage.

Another potential natural influence is tissue suffocation by mucilage produced by filamentous algae (Mistri & Cecherelli 1993). We detected only a minor degree of mucilage in some populations, and this could be the cause for the observed pale spots of tissue with smaller polyps. However, occurrence of mucilage often coincided with fishing lines that were in contact with these branches. Alternatively, pale colouring may not always indicate damage, as other causes (e.g. nutrition) may contribute.

In contrast, recent injuries, usually in the form of denuded skeleton that had not yet been colonized by epibionts, were obvious signs of human activity. Our observations suggest that these injuries were caused by direct mechanical contact with hard objects such as anchor lines, ropes connecting lobster pots, gill nets placed across gorgonian populations and lost monofilament lines. The destructive potential of the latter should not be underestimated. The strength and lifetime of a nylon fishing line is remarkable, and

a single line can cause extensive damage, migrating through a population subject to waves and currents, and thus keep injuring many more colonies than the ones it is observed to be in contact with at a given moment (until it becomes permanently entangled). Only few detached colonies were found lying on the bottom, probably due to direct detachment by entangled fishing lines or anchor lines, or increased drag by epibionts.

Fecundity and balance of energy reserves

The results of the carbohydrate–protein–lipid analysis are within the range of values reported by previous studies in the same species (Rossi et al. 2006a, Gori et al. 2007). Unaffected female colonies have larger lipid reserves (probably stored in the gonads) than females with epibionts. The gonad counts revealed that unaffected colonies in both males and females produced more gonads per polyp in all 3 populations. They also showed a significantly larger quantity of gonadal tissue volume, which is likely the cause of the higher lipid concentrations in unaffected females.

Overall, the results therefore confirm that colonies that are colonised by epibionts following injuries are not able to invest as much into reproduction as unaffected colonies, which indicates a compensation response by allocating energy resources that would normally be used for reproduction into recovery of injured tissue (see Silveira & Van't Hoff 1977). Such a response could even overcompensate for growth of injured tissue, as has been observed in tropical gorgonians (Wahle 1983, Castanaro & Lasker 2003).

The comparison of the carbohydrate and lipid concentrations in first-, second- and third-order branches revealed another way of allocating additional energy to injury recovery. It is based on the gorgonians' capability of using a system of gastrodermally lined canals described by Bayer (1973) to distribute food particles among the polyps of the colony (Murdock 1978a,b). Despite the small sample size, the analysis of the data shows that proteins and carbohydrates in unaffected colonies are distributed uniformly through first-, second- and third-order branches, as shown by Rossi et al. (2006a). As hydrodynamics dictate that apical polyps are more efficient in capturing prey (Dai & Lin 1993), this confirms an efficient transport of food from apical branches to the more central parts of the colony (sensu Murdock 1978a, b), which reinforces the stem to keep up with peripheral growth (Cadena et al. 2010). In contrast, tertiary branches in

colonies affected by epibionts contained significantly lower protein levels. Injured colonies therefore appear to reduce the transport of food from apical polyps to central ones, in favor of investing the resources into recovery of injured tissue (or growth of new branches) rather than growth of the central part. Future research on the recovery of corals from injury may elucidate the energetic cost of different types and degrees of injury, as well as the mechanisms of energy distribution patterns and re-allocation of resources in an attempt to better understand recovery rates and unrecoverable injury thresholds.

Effect of SCUBA bubbles on brood care

The survival of soft coral populations exposed to human impact depends not only on how injuries affect growth and reproduction, but also on how human impact affects recruitment. Given ca. 70 000 divers per year in each, the Medes Islands and Cap de Creus (see Lloret 2003b, Coma et al. 2004) and their tendency to concentrate on the most popular sites, effects surely surpass typical carrying capacities recommended for other sites (5000 to 6000 guided dives per year in Eilat; Zakai & Chadwick-Fuhrman 2002). It can thus be postulated that even subtle effects can lead to noticeable impact.

In this light, the observation that 3 to 4 exhalations of 1 diver are sufficient to remove virtually all eggs from the branches of this surface-brooding species is relevant to conservation and management. Arches, walls and overhangs are the preferred habitat of *Paramuricea clavata* within recreational SCUBA depths, so bubbles from divers passing below colonies may remove the eggs prior to fertilization. As the branches are the optimum location in the path of the current that transports sperm released by nearby male colonies, this could reduce fertilization considerably. Further research should focus on a detailed description of the reproductive cycle to identify and predict vulnerable periods. In the meantime, we suggest that this should be considered in management plans, as this impact type may be considerably mitigated by simply educating divers not to exhale below corals.

CONCLUSIONS

This study demonstrates that injuries in *Paramuricea clavata* through recreational and commercial fishing as well as SCUBA diving are widespread in shallow-water populations and pose a more immi-

ment threat than climate-related impact, by reducing their reproductive success, growth rate and thus resilience in all but strictly protected and well-enforced areas. Apart from deviating resources for reproduction into tissue recovery, this species appears able to adjust its intra-colonial food transport so that apical branches heal quicker. The patchy distribution of the populations and the small-scale spatial variability of human impact suggest that easily applicable mitigation measures could be put into place, such as habitat mapping (which would allow the application of nets with higher precision and minimize contact), avoidance of anchoring and cast fishing over coral populations, and briefing divers to not touch or swim underneath gorgonians.

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