

TOXICITY AND PALATABILITY OF GORGONIAN (OCTOCORALLIA: GORGONACEA) EXTRACTS TO GOLDFISH (*CARASSIUS AURATUS* (LINNAEUS, 1758))

Nigel K.C. Goh* and L.M. Chou

Department of Biological Sciences, National University of Singapore, Kent Ridge, Singapore 119260

Abstract

Eight of ten gorgonian extracts tested were toxic to goldfish; toxicity increased with exposure time and concentration. One of the two non-toxic extracts, i.e., of *Euplexaura* cf. *pinnata* caused agitated, nervous, behaviour in goldfish. When presented to goldfish, pellets loaded with extracts at concentrations equivalent to those in live gorgonians were rejected between 10% (in *Subergorgia suberosa* and *Echinogorgia* sp. C) to 90% (in *Ctenocella* (*Umbracella*) cf. *umbraculum*) of the time. Extracts of *Echinogorgia* sp. A and *Euplexaura* cf. *pinnata* were not rejected at this natural concentration. Increased loading with gorgonian extracts led to increased immediate rejection; at 5x 'natural' concentration, the lowest rate of rejection was seen in *Junceella* (*Dichotella*) cf. *gemmaea* (70%). With one exception, extract-loaded pellets decreased in their ability to deter feeding with time; in *Echinogorgia* sp. C at 'natural' and 2x 'natural' concentration, initial pellet rejection rates were lower than rejection rates after two minutes, indicating regurgitation of pellets that had been swallowed initially. The palatability of gorgonian extracts in Singapore is comparable to that seen in Caribbean gorgonians.

Introduction

Interest in the biological activities of secondary metabolites from marine organisms is increasing, not just for the pharmaceutical industry (Fenical 1997), but also to assess the ecological significance of such compounds (Hay 1996). Gorgonian bioactivity has generated much interest in recent years (Goh and Chou 1998a), with many compounds, novel structures and bioactivities being reported upon. The biological activity of gorgonian (Octocorallia: Gorgonacea) corals from Singapore only began to be studied in recent years (Goh 1996; Goh and Chou 1998b), and is currently still at the preliminary screening stage. Goh and Chou (1998b) presented data on the toxicity of gorgonian extracts towards lower organisms

(bacteria, fungi, invertebrates). The present study focusses on the effects of gorgonian extracts on a vertebrate (*Carassius auratus* (Linnaeus, 1758)) system.

Vertebrates possess more complex enzymatic and physiological systems than invertebrates and micro-organisms. It is, therefore, likely that screening against a vertebrate system would detect compounds not detectable in screens of invertebrates or micro-organisms. Besides being easily available, goldfish (*C. auratus*) have been used widely in tests of toxic extracts (Green 1977; Bakus 1981; Gerhart 1984; McCaffrey and Endean 1985; Thomson *et al.* 1985). Relative toxicities can therefore be compared. The use of the freshwater goldfish may seem illogical as a test organism for extracts of marine origin. Its use,

* Present address: Centre for Natural Product Research, 59A Science Park Drive, The Fleming, Singapore Science Park, Singapore 118240. Tel: (65) 871 9711, Fax: (65) 773 7072, E-mail: nigelgoh@post1.com

however, actually reduces uncertainty in the assay as a freshwater fish would not be likely to possess co-evolved resistance to either gorgonian toxins or feeding deterrents (Sammarco and Coll 1988). A negative result for toxicity to a marine fish occurring naturally with gorgonians might be false due to toxin resistance, even though toxic compounds are present.

Toxicity and palatability are known to be two separate responses often unrelated to each other as far as the compounds responsible for each of these responses are concerned (Pawlik 1993). Despite high fish predation rates on the marine invertebrates of tropical coral reefs (Bakus 1981), gorgonians are eaten rarely (Randall 1967; Bakus 1981; Gerhart 1984; Pawlik and Fenical 1992). This also appears to be true of Singapore reefs. In this study, both toxicity and palatability of gorgonian extracts were tested to identify gorgonian species possessing biologically-active compounds. Besides being able to detect bioactive compounds distinct from those active in toxicity assays, data from palatability tests also provide preliminary information on the possible ecological function of these metabolites as a deterrent against predation, and provide clues that may elucidate the action of the toxic metabolites.

Materials and methods

Collection and extraction

Ten common gorgonian species, identical to those assayed in Goh and Chou (1998b) were tested for bioactivity: i.e., *Subergorgia suberosa* (Pallas, 1766) and *Subergorgia mollis* (Nutting, 1910) (Subergorgiidae); *Acabaria robusta* (Shann, 1912) (Melithaeidae); *Junceella* (*Dichotella*) cf. *gemmacea*, *Ctenocella* (*Umbracella*) cf. *umbraculum* and *Ctenocella* (*Umbracella*) sp. A (Ellisellidae); *Euplexaura* cf. *pinnata*, *Echinogorgia* sp. A, *Echinogorgia* sp. C and *Echinogorgia* sp. E (Plexauridae). Descriptions of all the species collected can be found in Goh and Chou (1996). All colonies were hand-collected (each species in separate, labelled zip-loc bags) using SCUBA, frozen immediately using liquid nitrogen, then transported in ice to be stored at –

20°C in the laboratory. Frozen samples were exhaustively extracted using acetone; the latter was subsequently removed at reduced pressure using a rotary evaporator, leaving a viscous crude extract.

Extract toxicity

Goldfish (*Carassius auratus*) (5–6 cm total length) used in this bioassay were obtained commercially and allowed to acclimate to laboratory conditions for 2–3 days. Six fish were then put, in random order (using a random number table), into each of ten test aquaria containing 3000 ml of dechlorinated water and the test extract. Gentle aeration was maintained throughout the duration of the experiment. Four concentrations (2.5, 5, 7.5 and 10 mg·ml⁻¹) of each extract were tested. A control using the same volume of dechlorinated water was also set up. Mortality was recorded after acute (6 hr) and chronic (24 hr) exposure to the test solutions. Analysis was based on the Reed-Muench method (Teng 1993).

Extract palatability

Six goldfish were transferred from larger holding tanks and randomly (as for extract toxicity tests) distributed into tanks containing 5000 ml of dechlorinated water with continuous gentle aeration. Fish were allowed to acclimate to conditions in the tanks for 4–6 days before experiments were begun. During this time, they were subjected to a regular regime of daily feeding (aeration turned off) and a change of water every two days. Feed pellets were dropped into the tanks one at a time to pre-condition the fish to the palatability experiments. Commercial pelleted fish feed (Tetra Goldfish Feed, Tetrawerke, Germany) was used for both normal feeding as well as for the palatability experiments.

Feed pellets were prepared by soaking in freshwater for a total of ten minutes, with 3–4 changes of water. This was to minimise the odour of the feed that could mask any active chemicals present in the extracts. Pellets were air-dried before loading with extracts. Groups of ten feed pellets were laced with gorgonian extracts at 1x, 2x, 5x, and 15x the 'natural' concentration. The

'natural' concentration was obtained by incorporating into each pellet the amount of crude extract that would be found in a corresponding weight of gorgonian tissue. Pellets were dropped into the experimental tank one at a time, and the immediate (whether the pellet was spat out; all pellets were initially 'mouthed') and subsequent (total number of pellets eaten after one and two minutes) responses were scored. This was carried out for each test solution and control. Controls consisted of untreated feed pellets (only rinsed in freshwater to remove odour, as above), pellets treated with equivalent volumes of 20% acetone, and pellets treated with artificial seawater. All pellets were air-dried before feeding to the fish. Experiments were conducted at the normal feeding times of the fish, and under the same conditions to minimise the effect of unknown variables. Untreated feed pellets were interspersed among the treated pellets to minimise false negatives due to anticipation by the fish and to reduce the possibility of 'learned aversion' (Coll 1992). Experiments were discounted if the fish rejected the untreated feed pellets.

Results

Extract toxicity

No mortality was recorded in the control, in *Acabaria robusta* and *Euplexaura* cf. *pinnata* even after 24 hours at 10 mg·ml⁻¹ (Fig. 1A and B). Toxicity of extracts from the other eight species increased with exposure time and concentration for both acute and chronic exposures. After six hours, at 2.5 mg·ml⁻¹, both subergorgiid extracts (*Subergorgia suberosa* and *Subergorgia mollis*) did not kill any fish. *S. suberosa* was significantly more toxic than *S. mollis* at 5 mg·ml⁻¹ and 7.5 mg·ml⁻¹ (50% and 100% compared with 0% and 14%, respectively). The difference in mortality between *S. suberosa* and *S. mollis* was less pronounced at 10 mg·ml⁻¹ (100% compared with 80%, respectively). A similar (but more pronounced) pattern was seen after chronic exposure (Fig. 1B). After 24 hours, the extract of *Ctenocella (Umbracella)* cf. *umbraculum* caused 33% and 100% mortality in *Carassius auratus* at

2.5 mg·ml⁻¹ and 5 mg·ml⁻¹, respectively (Fig. 1B). This compares with 0% and 33% mortality for *Ctenocella (Umbracella)* sp. A at 2.5 mg·ml⁻¹ and 5 mg·ml⁻¹, respectively. As a family, the Plexauridae (*Echinogorgia* spp. A, C, E, and *Euplexaura* cf. *pinnata*) was less acutely toxic to *C. auratus* than the subergorgiids or the ellisellids, especially when comparing higher extract concentrations (Fig. 1A). This was most pronounced in the acute assay: at 10 mg·ml⁻¹, goldfish mortality in the plexaurid extracts never exceeded 29% (in *Echinogorgia* sp. C), in contrast to 86% mortality in *S. mollis* (the least toxic of extracts from other families at this concentration). After 24 hours (Fig. 1B), at 10 mg·ml⁻¹, the distinction was still evident, though not as pronounced, with 100% mortality seen in non-plexaurid extracts compared to a range of 33–91% for the three plexaurid species. At the intermediate concentrations (5 mg·ml⁻¹ and 7.5 mg·ml⁻¹), chronic toxicity of *Echinogorgia* spp. A and C extracts exceeded that of *S. mollis*; the other plexaurid species showed at most 50% mortality.

Extract palatability

All control pellets (rinsed only, rinsed and soaked in 20% acetone, rinsed and soaked in artificial seawater) were eaten by the goldfish. For all extracts, increased loading led to increased (or equivalent) immediate rejection (spitting out immediately after mouthing) of feed pellets (Fig. 2). The percentage range of pellet rejection at a 'natural' loading concentration was between 0% (no inhibition; *Echinogorgia* sp. A and *Euplexaura* cf. *pinnata*) and 90% (*Ctenocella (Umbracella)* cf. *umbraculum*). *Echinogorgia* sp. A — and *Euplexaura* cf. *pinnata* — loaded pellets were all accepted at 'natural' concentration, but at 2x the 'natural' concentration, rejection rates for both extracts increased to 90%. Rejection rates for all other extracts at 2x 'natural' concentration were between 10% (*Echinogorgia* sp. C) and 100% (*C. (Umbracella)* cf. *umbraculum*). At a 5x 'natural' concentration, the lowest rejection rate was seen in *Junceella (Dichotella)* cf. *gemmacea* (70%). All extracts except that of *J. (Dichotella)* cf. *gemmacea* (70%) produced 100% rejection rates at a 15x 'natural' concentration.

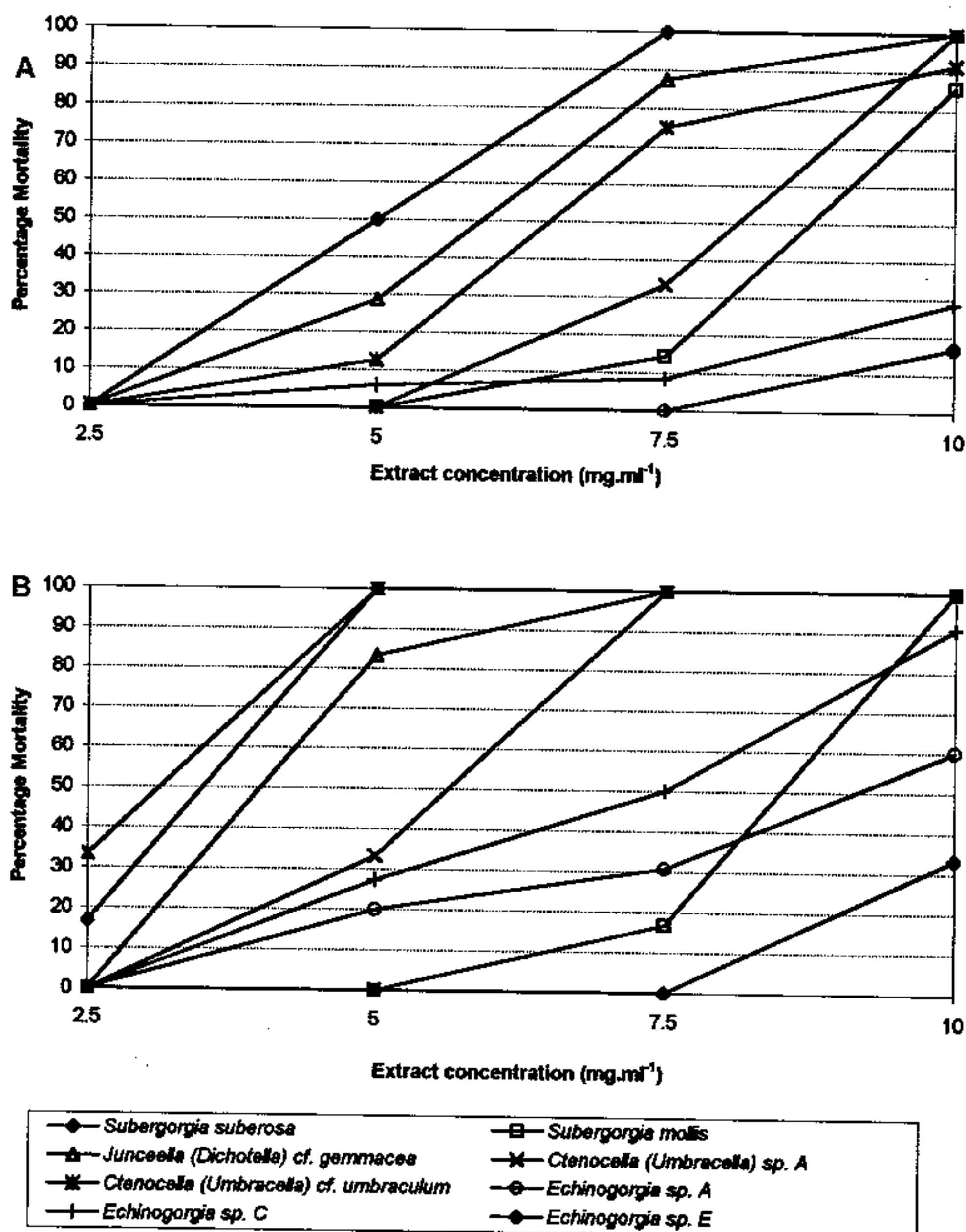


Fig. 1. Percentage mortality of *Carassius auratus* to various concentrations of crude gorgonian extracts after A, acute (6 hrs) and B, chronic (24 hr) exposure.

Note: 1. No mortality was recorded in *Acabaria robusta* and *Euplexaura cf. pinnata* extracts at all concentrations for both acute and chronic exposure.

2. No mortality was recorded in the control using dechlorinated water.

After one minute (Fig. 3), all pellets loaded at 'natural' concentration were eaten (except of *Ctenocella (Umbracella) cf. umbraculum* and *Echinogorgia sp. C*, where 80% and 60%, respectively of the pellets remained unconsumed). Loading at 2x the 'natural' concentration with extracts of *Subergorgia suberosa*, *Subergorgia mollis*, *Junceella (Dichotella) cf. gemmacea*, and *Echinogorgia sp. E* did not prevent their complete consumption after one minute. For all extracts at the 5x and 15x 'natural' concentration, at least

80% of pellets remained unconsumed after one minute.

Two minutes after the pellets loaded at the 'natural' concentration were offered to the goldfish (Fig. 4), 60% and 81% of them loaded with *Echinogorgia sp. C* and *Ctenocella (Umbracella) cf. umbraculum*, respectively, remained unconsumed. All other extracts failed to deter feeding at this loading concentration. Extracts of *Subergorgia suberosa*, *Subergorgia mollis*, *Junceella (Dichotella) cf. gemmacea*,

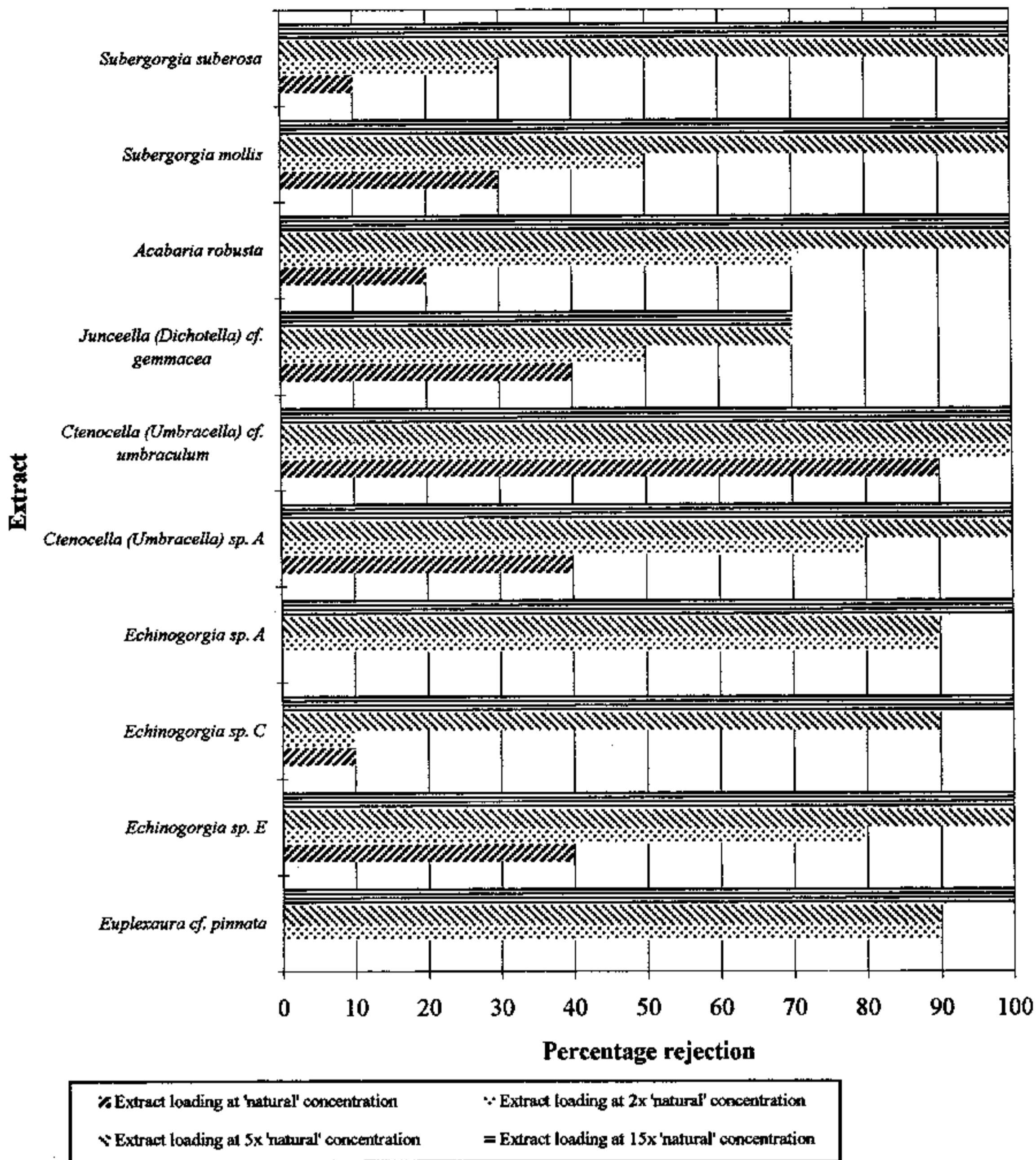


Fig. 2. Percentage of feed pellets rejected immediately after mouthing by *Carrassius auratus*.

Note: There was no rejection of any of the control pellets.

Ctenocella (Umbracella) sp. A, and *Echinogorgia sp. E* did not deter feeding upon pellets loaded at 2x the 'natural' concentration. At a loading of 5x the 'natural' concentration, all extracts deterred pellet consumption to some degree, with the lowest deterrence seen for *J. (Dichotella) cf. gemmacea* (50% pellets unconsumed). At 15x the 'natural' concentration, the lowest deterrence was seen in the *S. suberosa* extract (60% pellets unconsumed).

Discussion

Extract toxicity

The possibility that certain tanks contained fish that were more healthy is unlikely since both the selection of fish from the holding tank, and the choice of which experimental tank to place it in were random. In general, mortality was dependent on exposure concentration and duration (Fig. 1A

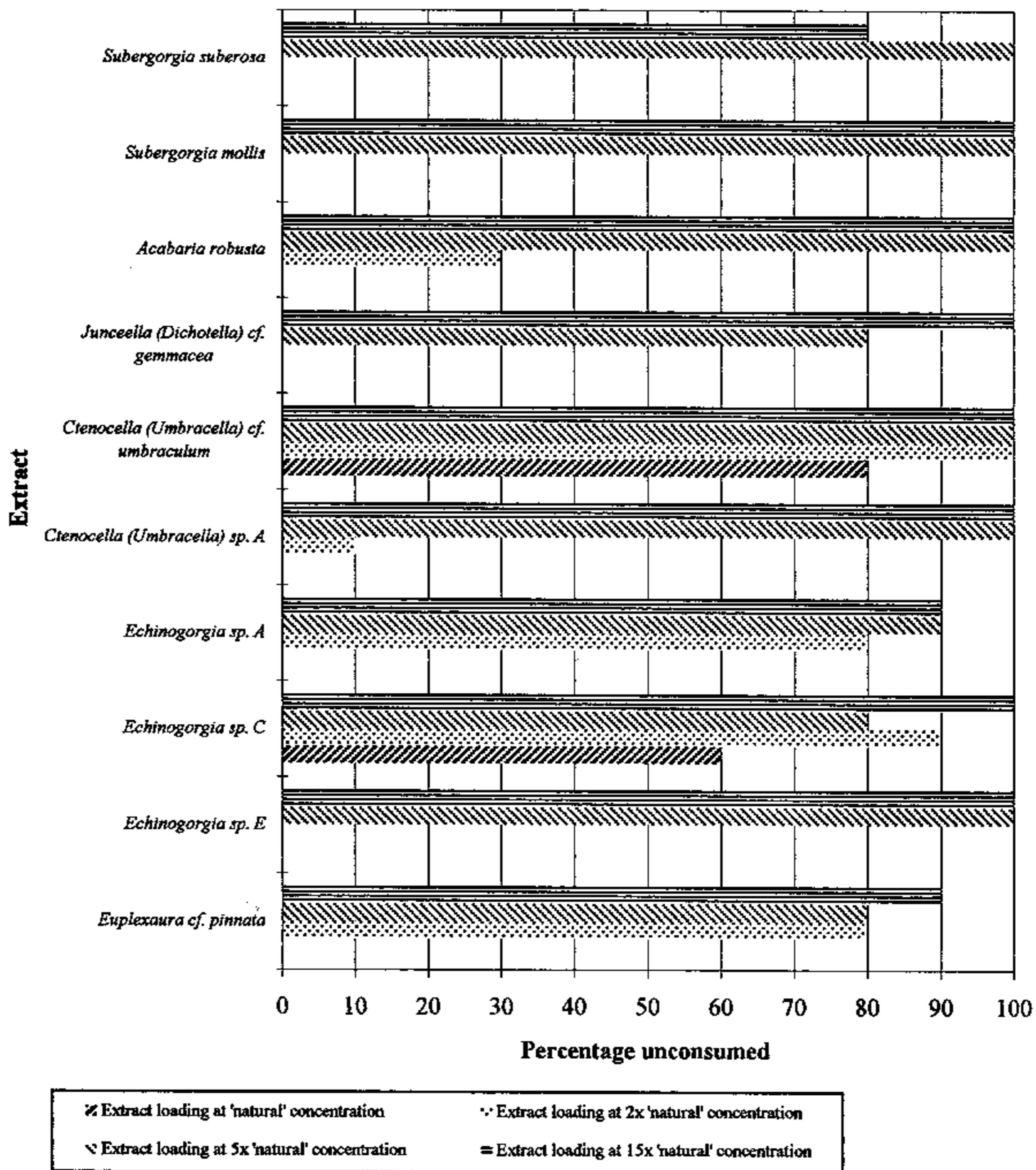


Fig. 3. Percentage of feed pellets unconsumed by *Carassius auratus* after one minute.
 Note: All control pellets were consumed.

and B), with generally sigmoidal curves which shifted to the left with increased (6 hours compared with 24 hours) exposure duration.

Although extracts of both *Acabaria robusta* and *Euplexaura cf. pinnata* did not kill any goldfish even after 24 hours, there were observable differences in fish behaviour between the two extracts. While goldfish in the former behaved normally (with respect to control animals observed simultaneously), the latter extract elicited agitated, nervous, behaviour. Sudden

bursts of swimming movements were frequent. Garcia-Alonso *et al.* (1993) have observed such excitatory behaviour in *Poecilia reticulata* Peters, 1860 exposed to gorgonian mucus and aqueous extracts. A similar response was also observed in the mosquito fish *Gambusia affinis* (Baird and Girard, 1853) when exposed to the cholinesterase inhibitor Iatruinculin A (Neeman *et al.* 1975). Intra-family variation in percentage mortality elicited by the different extracts was high in both acute and chronic exposures (Fig. 1A and B). Even

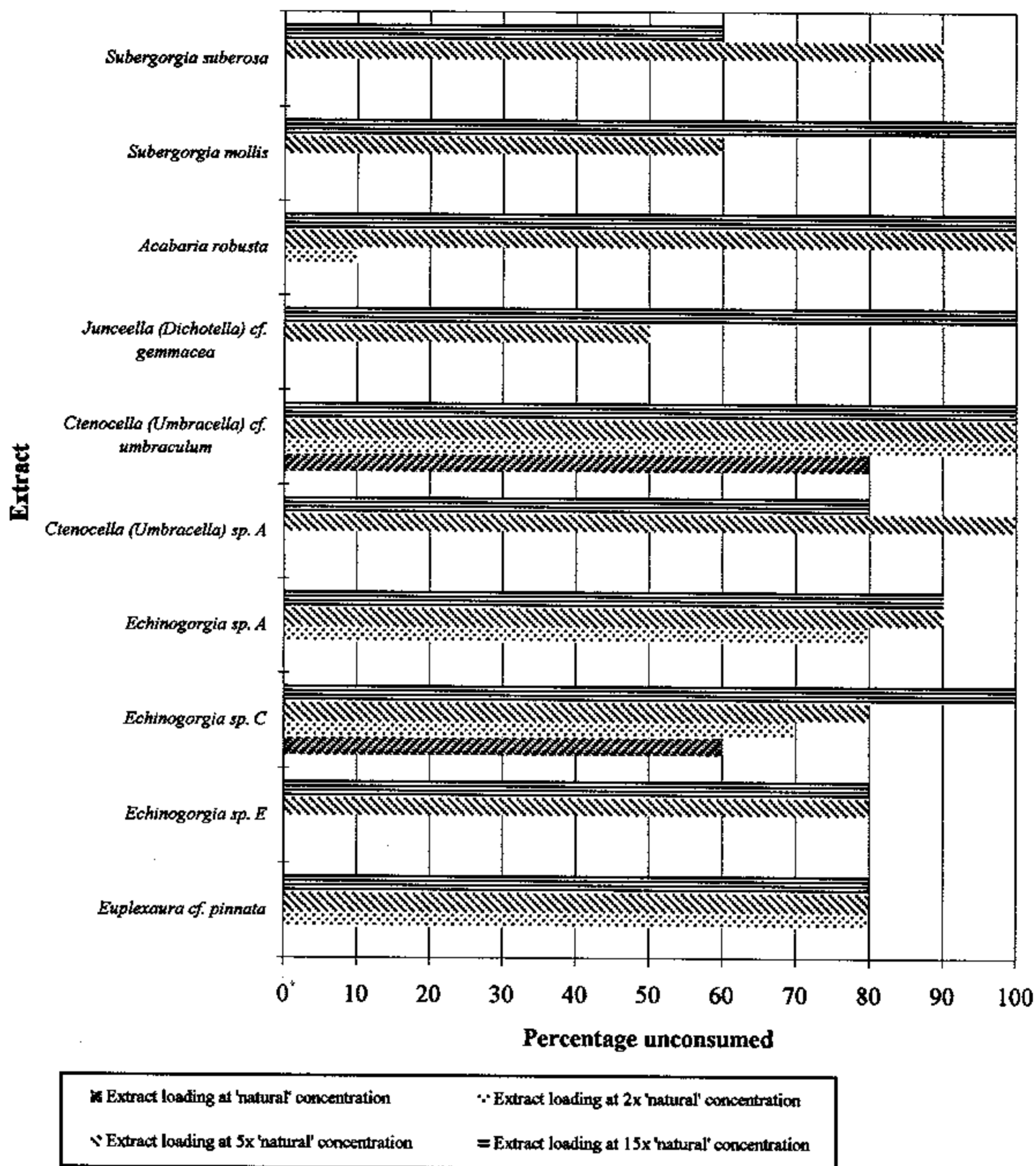


Fig. 4. Percentage of feed pellets unconsumed by *Carassius auratus* after two minutes. Note: All control pellets were consumed.

at the generic level, *Subergorgia* and *Ctenocella* showed significant differences in toxicity between species. Consequently, no patterns distinguishing different families were apparent, except that the plexaurid extracts appeared to be less toxic than those of the Subergorgiidae and Ellisellidae. Most reports of bioactivity in gorgonians (Goh and Chou 1998a) have been from extracts of Caribbean plexaurids. This may indicate inherent bioactive metabolites synthesised by members of this family, although it is more likely that this is a

reflection of the dominance (in terms of abundance and ubiquity) of the Plexauridae in the Caribbean (Bayer 1961), as well as the fact that most work on gorgonian natural products has been from material collected in that region. If the latter is true, then the bioactivity reported here from non-plexaurid gorgonians represents a promising, but as yet under-exploited chemical potential.

Only broad comparisons can be made between results of the present study with previous reports (of goldfish toxicity) because of differing

extraction protocols and the degree of extract purification. These comparisons are, however, necessary to ascertain the potential applicability of the bioactivity, and to direct future research on the biochemistry, pharmacology, and chemical ecology of Indo-West Pacific gorgonians. Thomson *et al.* (1985) reported upon goldfish toxicity using purified / partially purified sponge metabolites at concentrations of 0.001–0.01 mg·ml⁻¹ (10–100 µg·ml⁻¹). A partially purified toxin from *Halichondria* sp. killed Mosquito fish at 0.001 mg·ml⁻¹ (10 µg·ml⁻¹) (McCaffrey and Endean 1985). In the present study, the test concentrations were at least 250 times higher, ranging from 2.5 to 10 mg·ml⁻¹. The distinction between the use of wet crude extracts and purified metabolites must be noted in these comparisons. Extract potency in the present study compares well with a crude aqueous extract from the Caribbean gorgonian, *Plexaura homomalla* (Esper, 1792): Gerhart (1984) recorded death in his goldfish assay at a concentration equivalent to 50 mg·ml⁻¹ (5 g tissue in 100 ml distilled water).

Extract palatability

Pellet rejection due to satiation can be discounted in these experiments since control pellets offered between extract-loaded pellets were accepted readily and eaten. This experimental design also minimised the tendency for fish to associate pellets with unpalatability without actually tasting them, a phenomenon, described as 'learned aversion' (Bakus 1981; Gerhart 1984) that could occur if all ten extract-loaded pellets were given consecutively. The pronounced (compared with other extracts tested) increase in deterrence with increased concentration in *Echinogorgia* sp. A and *Euplexaura* cf. *pinnata* (Fig. 2) emphasizes the different responses between different extracts. In this instance, a threshold concentration between the 1x and 2x the 'natural' concentration was detected by the assays.

Sammarco and Coll (1988) identified four categories of fish responses to secondary metabolites extracted from octocorals: olfaction, palatability, emesis, and any combination of these. Noting the responses of the goldfish immediately, and after one and two minutes, enabled inferences

to be made about the categories of responses in relation to the different extracts. The fact that none of the pellets was ignored, i.e., all were 'mouthed', suggests either that olfaction is not an important means of unpalatability detection in goldfish, or that the extracts were odourless. In general, extract-loaded pellets left in the aquarium became more palatable with time. This may indicate that the deterrent compounds are water-soluble and gradually leached into the surrounding water. It is interesting to note that the extract of *Echinogorgia* sp. C was initially palatable at a 'natural' concentration (Fig. 2: only 10% of pellets spat out immediately) but after two minutes, 60% of the pellets were left in the water (Fig. 4). This suggests that this extract did not have a strong negative effect on palatability, but later caused regurgitation of the swallowed pellets. Gerhart (1984) has reported emesis caused by prostaglandins (derived from gorgonians) loaded onto feed pellets. As in the present experiment, there was no initial rejection of pellets.

Pawlik *et al.* (1987), working in the Caribbean, also conducted palatability tests involving a large number of gorgonian extracts. Although the assay fish used, assay methodology, and treatment of feed pellets differed, a comparison between that study and the present one allows some generalisations to be made. Since both studies tested a relatively large number of extracts, this gives an approximate estimate of the relative palatability of gorgonians from Singapore, as compared to those from the Caribbean. Pawlik *et al.* (1987) tested 37 extracts, and showed that 51% were 'highly unpalatable' (one or less of five pellets eaten; i.e., 20% or less eaten), 11% were 'moderately unpalatable' (two to three pellets eaten; 40–60% eaten), and 38% were 'palatable' (two or more pellets eaten; 80% or more eaten). In comparison, at the 'natural' concentration, 10% of Singapore extracts were 'highly unpalatable', using the definition of Pawlik *et al.* (1987), 40% were 'moderately unpalatable', and 50% were 'palatable'. At 2x the 'natural' concentration, 50% were 'highly unpalatable', 40% were 'moderately unpalatable', and only 10% were 'palatable'. At the two highest concentrations, 90% were 'highly unpalatable', 10% were 'moderately unpalatable', and none were 'palatable'. Since the present study

consisted of ten rather than five replicates, the range for 'moderately unpalatable' was extended to between 20% and 80%. These results indicate that the palatability of gorgonian extracts from Singapore is comparable to that of extracts from the Caribbean. What remains is to test these extracts in the field against generalist fish predators that could feed on gorgonians to determine if these unpalatable compounds have an ecological function.

Conclusions

Results from the present study were combined with those from Goh and Chou (1998b) to elucidate broad-based trends in the bioactivity of Singapore gorgonians (Table 1). The unique toxicity of ellisellid extracts against yeast cells has already been noted (Goh and Chou 1998b). Extracts from the Plexauridae (mean number of '+s = 6.5) were generally less toxic than the Subergorgiidae (12.5) and the Ellisellidae (13.3). Plexaurid extracts were, however, more unpalatable than extracts of the other two families (mean number of '+s = 7.8; compared with 5.0 and 7.0 for the subergorgiid and ellisellid extracts, respectively). The plexaurid *Euplexaura* cf. *pinnata* underscores this distinction: the extract was non-toxic in all assays except in the 24 hour brine shrimp toxicity assay where it only scored '+'; in the palatability studies, this extract scored '+++' in all three categories. This reiterates the fact that toxicity and palatability are not directly related (Schulte and Bakus 1992). No correlation could be found between toxicity and palatability of soft corals (La Barre *et al.* 1986; Sammarco *et al.* 1987), and toxicity could not explain the feeding preferences of the soft coral predator *Chaetodon melannotus* Bloch and Schneider, 1801 (Alino *et al.* 1988). Pawlik (1993), in his review of marine invertebrate chemical defenses, was of the opinion that the toxicity of metabolites had little to do with the capacity of compounds to deter predators. Under natural conditions, an unpalatable chemical might have either an equal or superior survival value compared to a toxic compound, although they might be ranked differently based on laboratory assay results (Schulte and Bakus 1992). While these observed

differences in patterns of toxicity and palatability may suggest a variety of chemical defenses related to different strategies employed by different families, at the moment this remains conjecture which can only be confirmed by corroboration from laboratory assays using naturally-occurring fish, and from field studies.

Testing a large number of extracts against a broad spectrum of assay organisms provides several advantages. Trends in bioactivity (suggesting an underlying biochemical cause) within and between taxonomic families can be revealed. This provides valuable leads, both to the pharmacologist (to focus attention on extracts from 'promising' families), and to the chemotaxonomist (to follow up on these apparently taxonomically-delineated patterns). Each assay organism possesses unique sensitivities to different compounds and, using a variety of assay organisms enables a larger number of compounds with bioactivity to be detected. A third advantage of this strategy is that extracts with selective bioactivity, e.g., as in *Euplexaura* cf. *pinnata*, can be identified; this would not be possible if extracts are only tested in one assay. Such extracts have high priority in the development of pharmaceuticals, where only compounds with highly specific and selective targets are selected as potential lead compounds. Compounds with non-selective bioactivity are generally not pursued as pharmaceutical leads.

Thomson *et al.* (1985) showed that tests employing organisms such as non-marine goldfish were useful in identifying metabolites with potential activity against marine organisms. At the same time, muricins from the gorgonian *Muricea fruticosa* Verrill (Bandurraga and Fenical 1985) inhibited diatoms (anti-fouling activity), yet did not possess cytotoxic, ichthyotoxic, or anti-microbial activity. To provide an insight into the possible biological and ecological roles of the metabolites present, more ecologically-oriented bioassays need to be employed. In the past, chemists studying marine natural products have focussed on the identification of novel classes of bioactive compounds, with either little or no regard for the ecological function of the metabolites. Ecological studies, on the other hand, have ignored or only provided anecdotal

Table 1. Summary and scoring of bioactivities of the ten gorgonian extracts. Mean values for the different parameters were calculated, and scores were worked out according to the scheme below: - : no inhibition, toxicity, or deterrence; + : value for inhibition, toxicity, or deterrence falls between 0 and 75% of the mean value; ++ : value for inhibition, toxicity, or deterrence falls between 75% and 125% of the mean value; +++ : value for inhibition, toxicity, or deterrence falls above 125% of the mean value.

FAMILY	SPECIES	Bacteria (inhib. diam.)		Yeast (inhib. diam.) diam.)	Fungi (inhib. diam.)	Brine Shrimp Toxicity (at 50 mg·ml ⁻¹)			Goldfish Toxicity (LD ₅₀)			Feed Palatability (ED ₅₀)	
		Gram +ve	Gram -ve			6 hr	24 hr	6 hr	24 hr	24 hr	6 hr	1 min.	2 min.
SUBERGORGIIIDAE	<i>Subergorgia</i>	++	+++	-	-	+++	+++	++	+++	+	++	++	++
	<i>suberosa</i>												
	<i>Subergorgia mollis</i>	+++	+++	-	-	+++	++	++	+				
MELITHAEIDAE	<i>Acabaria robusta</i>	++	-	-	-	-	+++	-	-	++	++	++	++
	<i>Junceella</i> (<i>Dichotella</i>) cf. <i>gemmacea</i>	++	+++	+++	-	+	+	++	+++	++	+	++	++
ELLISELLIDAE	<i>Ctenocella</i> +++ (<i>Umbracella</i>) cf. <i>umbraculum</i>	-	+++	-	-	+	+	+++	+++	+++	+++	+++	+++
	<i>Ctenocella</i> (<i>Umbracella</i>) sp. A	++	-	+++	-	+++	+++	+	++	+++	++	++	++
	<i>Echinogorgia</i> sp. A	++	-	-	-	-	+	+	+	+++	+++	+++	+++
PLEXAURIDAE	<i>Echinogorgia</i> sp. C	++	-	-	-	+	++	+	+	+	+	+	+
	<i>Echinogorgia</i> sp. E	++	+++	-	-	+++	+++	+	+	+++	+++	+++	+
	<i>Euplexaura</i> cf. <i>pinnata</i>	-	-	-	-	-	+	-	-	+++	+++	+++	+++

information on the involvement of chemical metabolites in the ecology of coral reef organisms. In addition to leads for possible pharmaceutical applications, this study provides the groundwork from which ecologically relevant compounds can be isolated and characterised as part of more in-depth interdisciplinary studies involving marine natural products chemistry and coral reef ecology.

Acknowledgements

This work was supported by research grant RP960303/A from the National University of Singapore Academic Research Fund. The Reef Ecology Study Team (Department of Biological Sciences, NUS) assisted in field collections.

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