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DIPLOMARBEIT

„TOXICITY OF CORAL-ASSOCIATED *GOBIODON* SPECIES (PISCES: GOBIIDAE) AND ITS EFFECT ON PREDATOR BEHAVIOR AND PREY SURVIVAL“

Barbara Gratzer

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INHALTSVERZEICHNIS

IM WEITEN MEERE MUSST DU ANBEGINNEN!
DA FÄNGT MAN ERST IM KLEINEN AN
UND FREUT SICH, KLEINSTE ZU VERSCHLINGEN;
MAN WÄCHST SO NACH UND NACH HERAN
UND BILDET SICH ZU HÖHEREN VOLLBRINGEN.

GOETHE

Gewidmet sei diese Diplomarbeit jener Meeresschildkröte
der ich diesen Weg zu verdanken habe

1 EINLEITUNG

Biologische Toxine sind Produkte, welche zumeist schädlich für all jene Organismen sind, welche das Toxin selbst nicht produzieren (Cameron 1974), vor allem für nicht resistente Zielorganismen. Toxizität ist in den meisten Lebensräumen anzutreffen und spielt in vielen ökologischen Prozessen – bei Konkurrenzverhalten (Cameron 1974) und insbesondere bei Räuber-Beute-Beziehungen – eine wichtige Rolle. Forschungsdisziplinen aus den verschiedensten Themenbereichen befassen sich mit Toxizität, welche bisher bei einer Fülle von Organismen aus den verschiedensten systematischen Gruppen beschrieben wurde: Arthropoden wie Coleoptera, Formicidae, Acari und Myriapoda, sowie über 800 Amphibien-Arten mit Hautalkaloiden (Daly et al. 2005); im marinen Bereich die Porifera (Proksch 1994), Gorgonien (Pawlik und Fenical 1992, Epifanio et. al 1999, Koh et al. 2000), Steinkorallen (Gunthrope und Cameron 1990), Ascidien (Pisut und Pawlik 2002), sowie Echinodermaten (Bakus 1981, Lippert et al. 2004) und Fische (Pawlowsky 1927).

Innerhalb dieser Vielzahl an toxischen Tieren gibt es große Unterschiede hinsichtlich der chemischen Zusammensetzung und Funktion von Giften. Ihre Wirkung ist abhängig von geographischen Bedingungen und Jahreszeiten, sowie der vom Empfänger aufgenommenen Giftdosis (im Verhältnis zum Körpergewicht), der Form, in welcher Gift in einen Organismus eindringt (gasförmig, über die Verdauung oder auf indirektem Weg über die Haut zum Blut), und nicht zuletzt auch von der Empfindlichkeit des Empfängers. Letale Gifte, wie etwa Schlangengift, können zum Beispiel ihre Wirkung verlieren wenn sie nicht über den Blutkreislauf sondern über die Verdauung in den Körper gelangen (Pawlowsky 1927). Grundsätzlich sind Toxine auf keine bestimmte Körperregion beschränkt und können überall lokalisiert sein. Die Klassifizierung von Toxinen erfolgt primär über die Produktionsweise und Toxinabgabe (Halstead 1970). Man unterscheidet zwischen aktiv giftigen Tieren, welche mit Hilfe von Stechapparaten das Gift zuführen, und passiv giftigen Tieren, bei welchen die Giftigkeit eine Folge der chemischen Beschaffenheit von Geweben und Organen ist (Pawlowsky 1927). Bei toxischen Fischen (Klasse Teleostei; Knochenfische) unterscheiden Primor und Zlotkin (1975) neben aktiv (engl.: venomous) und passiv (engl.: poisonous) giftigen Fischen eine dritte Giftklasse: Ichthyokrinotoxine (Halstead 1970), bei welchen sich die Toxine in der Haut befinden.

Zu den ichthyokrinotoxischen Fisch-Familien zählen unter anderem Schleimaale (Myxinidae) (Lim et al. 2005), Welsartige (Siluriformes) (Manivasagan et al. 2009), Igelfische

(Diodontidae, Tetradontiformes) (Battley et al. 2008), Seifenbarsche (Grammistidae) (Sugiyama et al. 2005), Kofferfische (Ostraciidae, Tetradontiformes) (Thomson 1964) und Korallengrundeln (Gobiidae) (Hashimoto et al. 1974). Obgleich der mannigfaltigen Funktionen und Wirkungen von Toxinen ist festzuhalten, dass die Entwicklung eines Giftes keinem eindeutig beschreibbaren Vorgang folgt, sondern individuell zu betrachten und damit auch zu untersuchen ist (Williams et al. 2003).

Vor diesem Hintergrund toxischer Wehrsubstanzen, gilt diese Arbeit der Untersuchung der Toxizität von Korallengrundeln der Gattung *Gobiodon*, welche in Korallenriffen leben und einen hohen Spezialisierungsgrad aufweisen (Munday et al. 1997, Dirnwöber und Herler 2007). Dieser basiert auf einer hohen Bindungsaffinität zu ihrem Wirt, Steinkorallen der Gattung *Acropora*. Innerhalb dieser Beziehung zwischen Grundel und Koralle gibt es jedoch Unterschiede bezüglich der Lebensweise in einer Koralle, wobei zwischen Arten mit kryptischer Lebensweise und damit einhergehender angepasster Körperfärbung (meist dunkel) und zwischen weniger kryptisch lebenden Arten mit meist auffälliger Färbung (und Musterung) zu unterscheiden ist. Arten mit kryptischer Lebensweise sind insbesondere für Prädatoren (= Räuber) schwerer zugänglich und weisen vermutlich geringere Migrationsraten auf (Herler, pers. Kommentar). Vor allem wenn die Korallengrundeln mit einem Brutpartner zusammen leben, mit dem sie vermutlich monogam leben, vermeiden es die meisten Fische die Wirtskoralle zu verlassen (Wall und Herler 2009). Neben der monogamen Lebensweise besitzen *Gobiodon* Arten auch die Fähigkeit im Fall von Partnerverlust das Geschlecht in bi-direktionaler Richtung zu wechseln (Munday et al. 1998). Beide Strategien geben einen Hinweis darauf, dass die Fische die Koralle möglichst selten verlassen, vermutlich um großem Raubdruck zu entgehen. Aufgrund der vielfach spezialisierten Lebensweise ist anzunehmen, dass *Gobiodon* Arten stark von ihrer Wirtskoralle profitieren. Trotz des Schutzes, den sie durch ihre Koralle genießen, weisen Korallengrundeln Toxine in ihrer unbeschuppten Haut auf (Hashimoto et al. 1974). Die ersten histologischen und chemischen Untersuchungen über Beschaffenheit und Funktion des Hautgiftes liefert Hashimoto et al. (1974). Lassig (1981) beschäftigte sich ebenfalls mit der Wirkung der Toxine und stellte erstmals "bioassay"-Untersuchungen an, eine Methode mit der man die Giftwirkung einer Substanz an einem anderen lebenden Organismus testet. Ein Vergleich mit dem nahe verwandten und beschuppten *Paragobiodon echinocephalus* (Rüppell, 1830) zeigte, dass ausschließlich der Hautschleim von *Gobiodon* negative Auswirkungen auf die lokomotorischen Fähigkeiten von anderen Fischen hat und damit womöglich sowohl zur Verteidigung der Wirtskoralle gegenüber korallivoren Fischen, als auch zur Abwehr von anderen

korallenbewohnenden Fischen dient. Munday et al. (2003) entdeckten außerdem, dass eine Kohärenz zwischen Parasiten und deren Lokalisierung am Körper der Korallengrundel besteht, wobei diese sich auf die nicht giftigen Flossen beschränken. Schubert et al. (2003) untersuchten erstmals experimentell einen möglichen Zusammenhang zwischen Giftigkeit und Raubdruck (Prädation) mittels künstlich präparierten Nahrungspellets (mit und ohne *Gobiodon*-Hautsekreten) und konnte zeigen, dass Räuber ein Vermeidungsverhalten aufweisen. Des Weiteren stellten die Wissenschaftler einen Zusammenhang zwischen Toxizität und Färbung fest, wobei kryptisch-lebende und -gefärbte Tiere weniger giftig waren als auffällig gefärbte Arten. Ein möglicher Zusammenhang zwischen Toxizität und Prädatorpräferenz wurde aufgezeigt. Bisher wurden mögliche Effekte, welche die Toxizität eines Tieres auf das Räuberverhalten haben kann, üblicherweise mittels künstlich manipulierter Experimente (engl.: feeding bioassay) untersucht (Pawlik und Fenical 1992, Nagle und Paul 1998, McClintock et al. 1999, Pisut und Pawlik 2002, Mahon et al. 2003, Schubert et al. 2003). Im Feld der Toxikologie und der Ökologie von Toxinen sind naturnahe Experimente jedoch rar. Einige wenige Studien haben direkte Räuber-Beute-Interaktionen verschiedenster Tiergruppen direkt mittels Videoanalyse untersucht (Christensen 1996, Nemeth 1997, Mahjoub et al. 2008, Staudinger et al. 2011). Es gibt jedoch kaum direkte Beobachtungen des Verhaltens von Räubern gegenüber toxischen Beutetieren.

Um präzisere Informationen über *Gobiodon*-Räuber-Interaktionen und die Rolle der Toxizität zu erhalten, habe ich in meiner Diplomarbeit Videoexperimente durchgeführt. Diese sollten Aufschluss darüber geben, ob und von welchen Räubern Korallengrundeln generell gefressen werden und ob sie selbst dann gefressen werden, wenn andere, nicht giftige Beutefische verfügbar sind. Letzterer war der beschuppte und mit starken Flossenstrahlen bewehrte Riffbarsch *C. viridis*. Weiters soll dieses Experiment über Prädatorpräferenzen gegenüber unterschiedlichen *Gobiodon*-Arten Aufschluss geben und damit die Annahme untersuchen, ob ein Zusammenhang zwischen Giftigkeit bzw. Färbung von Korallengrundeln und entsprechender Prädatorpräferenz besteht. Um das Räuberverhalten besser interpretieren zu können, war es notwendig grundlegende Experimente zur Bestimmung der Toxizität zweier *Gobiodon*-Arten aus dem Roten Meer durchzuführen, wobei ich jeweils einen häufig vorkommenden Vertreter der kryptisch bzw. der auffällig gefärbten Arten wählte. Dadurch wurde zusätzlich ein Vergleich mit den *Gobiodon*-Arten in Japan (Hashimoto et al. 1974) und Arten im großen Barriereriff vor Australien möglich (Schubert et al. 2003). Unterschiede der Toxizität wurden mittels bioassay getestet, bei dem Effekte von im Meerwasser gelösten

Gobiodon-Hautsekreten auf das Verhalten einer sensiblen bioassay-Art (die Riffbarschart *Chromis viridis*) beobachtet wurden. Weitere wichtige Informationen, die für das darauf folgende Prädationsexperiment hilfreich waren, betrafen: (1) jene Zeitspanne die notwendig war bis eine Korallengrundel ihr gesamtes Toxin abgegeben hat (durch manuelle, mechanische Stimulation); (2) Kenntnisse über jene Zeit, innerhalb welcher das Toxin, einmal exprimiert, seine vollständige Giftigkeit beibehält, und (3) die Dauer, die eine Korallengrundel nach Abgabe der schädlichen Giftmenge zur Wiederherstellung einer toxischen Wirkung benötigt. Durch diese Informationen war es mir möglich verschiedene Ereignisse im Prädatorexperiment besser erklären zu können und entsprechende ökologische Interpretationen anzustellen. Damit liefert diese Arbeit einen wichtigen Beitrag zur Ökologie häufig vorkommender korallenassoziierter Meergrundeln und zum besseren Verständnis spezifischer Giftwirkungen bei hochspezialisierten Rifffischen.

2 ABSTRACT

Coral-dwelling gobies of the genus *Gobiodon* primarily live in scleractinian corals of the genus *Acropora*. Preferences of coral species are species-specific and highly variable among *Gobiodon*. The degree of association is possibly correlated with conspicuousness and crypticity of body colouration, respectively. An explanation for the close attachment to their host coral and other particular traits such as bidirectional sex-change, high mate fidelity and especially for the development of skin toxins is that predation risk is particularly high for small gobies. In the scope of my diploma thesis I compared two crinotoxic (skin toxins) coral gobies, *Gobiodon histrio* (Valenciennes, 1837), representative for a conspicuously coloured species, and *Gobiodon sp*.3 (in sensu Herler and Hilgers 2005) with cryptic colouration, with respect to toxicity levels and predator preferences. Toxicity was tested using a standardised bioassay method, in which a damselfish species, *Chromis viridis* (Cuvier, 1830), was employed for measuring loss of equilibrium times. It was revealed that both *Gobiodon* species are toxic with a significant difference at the beginning (Mann-Whitney-U-test: $p < 0.05$) but toxicity equally declines until 60 minutes after toxin release in both species - regardless of basic toxicity levels (ANCOVA: $p = 0.61$ for homogeneity of slopes). Predator preferences were studied after a suitable piscivorous predator – *Epinephelus fasciatus* (Forsskål, 1775) – was selected and its behaviour studied using video-analysis in an experimental aquaria. Both *Gobiodon* species and a non-toxic control (*C. viridis*) were offered to the predator and predator-prey interactions were recorded in approximately 9.5 hours trials. Video analysis revealed that *E. fasciatus* preferred the non-toxic control fish (*C. viridis*) over *Gobiodon*. Both coral gobies appeared to be potential prey of *E. fasciatus,* but gobies were repeatedly and quickly expelled after capture and were ingested only after a certain time of manipulation by the predator. *Gobiodon histrio* was slightly preferred over *G.* sp.3. With this work I have shown that *Gobiodon* has a great potential for the survival of predator encounters in the field due to proposed skin toxins. I suggest that their host coral may provide additional protection since some gobies may be consumed after repeated expel and that toxic deterrence appears to be more efficient than physical deterrence provided by body squamation and strong fin spines.

3 INTRODUCTION

3.1 Predator-prey interactions

Most toxic species occur in species-rich environments that comprise high rates of competition and strong predation pressure. One of the most diverse habitats in the world, the rainforest, occupies a high number of toxic species. Well-known representatives are frogs of the family Dendrobatidae, which contain lethal toxins in their epidermis and use aposematic signals as a warning for predators (Saporito et al. 2007). Other habitats comparable with rainforests concerning species richness, competition and enhanced predation are coral reefs, an ecosystem characterised by constrained food availability and shelter. Thereby interactions between predators and prey have a great impact on population dynamics, which is reflected in the numerous strategies developed by animals for efficient prey capture and defence against predators. Typically, a predator-prey interaction starts with the predator searching for prey, followed by encountering, attacking, capturing and, finally, ingesting the prey. Naturally, a prey would try to interrupt this course as soon as possible (Brönmark and Hansson 2000). Different strategies are used by prey to avoid capture: hiding, escaping in space or time or by behavioural, physical or chemical defence mechanisms (Pawlowsky 1927, Brönmark and Hansson 2000, Duffy and Hay 2001). The most common strategy used by both predators and prey is toxicity, either actively by venomous animals to harm others, or passively by possessing toxic body parts – a defence mechanism occurring frequently in areas with constantly high predation pressure (Beukers-Stewart and Jones 2004). This suggests that the ultimate cause of toxicity is natural selection under high intensities of predation (Bakus 1981). But toxicity also affects intra- and interspecific interactions and serves as avoidance of parasites, fouling, microbial activity, pathogens and infections (Mahon et al. 2003, Munday et al. 2003).

The evolvement of lethal toxins and resistance to them are an evolutionary paradoxon, even though toxicity occurs in a broad spectrum of organisms. On the one hand it is obvious that self-defence via production of a lethal toxin is an advantage since it enhances the prey's survival rate. On the other hand no selection for an increased resistance can take place, if predators do not survive attacks of toxic prey. But not only toxins, also other mechanisms such as distastefulness and alarm cues constitute a similar evolutionary enigma: distastefulness is a repellent effect without lethal consequences for the predator, whereas preys usually would not survive (Williams et al. 2003) and chemical alarm cues simply indicate predation threat to conspecifics of the sender without deterring predators (McCormick and Larson 2007). All these mechanisms

primarily suggest a selective advantage for the species since the affected species does not receive any direct benefits.

3.2 Ichthyotoxic fishes

Besides actively (venomous) and passively (poisonous) toxic fishes, ichthyocrinotoxic fishes (Halstead 1970) are specified, with the latter being distinguished regarding to cytological toxin production and release between (1) mucous secreting cells, typically occurring as goblet cells and (2) proteinaceous epidermal toxins (crinotoxins and venoms) that are produced by gland cells. Crinotoxic fishes tend to have reduced squamation and fishes with either type of toxin often adopt a sedentary, station-keeping, and sometimes cryptic mode of life. The crinotoxic products of gland cells can only be released after damage to the epidermis, leading to the assumption that they play a major role for the protection against other organisms (Cameron and Endean 1973) such as viruses, bacteria and fouling organisms (Lassig 1981), fungus, algae, settling invertebrate larvae or predators (Cameron and Endean 1973). About 50 species of 14 teleost families were reported to be crinotoxic (Halstead 1970) and some of these species are avoided by predators, suggesting that the toxins function as predation deterrent (Randall et al. 1971, Shiomi et al. 2000). Commonly, the toxins are capable of lysing both mammalian and bacterial cells similar to toxins from the bee venom (melittin), thereby intoxicating other fish while making the surrounding seawater foamy (Hashimoto 1979, Shiomi et al. 2000). They comprise great structural diversity (Sugiyama et al. 2005). Thomson (1964) observed that the introduction of a highly excited boxfish ((*Ostracion lentiginosus*) into an aquarium with other fishes often results in death of others, a result of pahutoxin (formerly: ostracitoxin). Boxin, a related ichthyotoxin from a trunkfish was described by Kalmanzon and Zlotkin (2000). Both toxins do not show any effect by a subcutaneous injection but achieve their ichthyotoxicity only upon delivery to the surrounding water suggesting the lethal effect on other fishes is mediated by externally allocated target sites, such as the gills. Crinotoxins were also found in flatfishes (Primor and Zlotkin 1975) and soapfishes (Grammistidae) (Randall et al. 1971), which release grammistin, a simple peptide, related to melittin (bee venom) and paradaxin (soles) (Shiomi et al. 2000). Apart from soapfishes, a clingfish (family Gobiesocidae) (Goldberg et al. 1988) and gobiids of the genus *Gobiodon* (Hashimoto et al. 1974) have been reported to secrete skin toxins possibly related to grammistin.

3.3 Biology of coral gobies (genus *Gobiodon*)

Coral gobies are highly specialised, because they show strong associations with coral colonies of the genus *Acropora* (Dirnwöber and Herler 2007, Munday et al. 1997). As many crinotoxic fishes, they lack squamation and live a sedentary mode of life. These fishes spend their entire postlarval life-span among branches of patchily distributed colonies and rarely move between them (Munday 2004, Feary et al. 2007, Wall and Herler 2009). This association seems to be generated by the habitat selection of previously pelagic larvae at or near the time of settlement to the reef (Munday 2001). Among species of *Gobiodon* the habitat specialisation degree is variable (Munday et al. 1997, Dirnwöber and Herler 2007) and association intensity with coral host as well as colour (ranging from conspicuously to cryptically coloured) are variable as well (Munday et al. 1999, Herler and Hilgers 2005). Typically, coral gobies form breeding pairs (Lassig 1977) and pairs often remain in the same host coral for most of their adult life (Munday 2004). They show high fidelity to their partners (Wall and Herler 2009) and are capable of bi-directional sex-change (Munday et al. 1998). The diet of *Gobiodon* spp. includes coral tissue, filamentous algae and copepods (Riedlecker & Herler 2009, Brooker et al. 2010).

It is apparent that coral gobies rely on their coral host for their entire post-larval life for many reasons: shelter, food and nest sites (Lassig 1981), suggesting that the coral host is highly advantageous for the survival of the gobies. This specialisation and relatedness on the host could be evidence for a high predation pressure in the surrounding environment outside the coral (Schubert et al. 2003). Movements outside the coral host may be particularly hazardous for coral gobies due to their small body size, and conspicuousness, their poor long-distance swimming ability and poor swimming speed (Lassig 1981), as well as their possible reduced visual acuity (Niedermüller, personal communication). A possible tool deterring predators may be the toxic skin secretions of *Gobiodon* (Schubert et al. 2003) since their toxins are ichthyotoxic and haemolytic (Hashimoto et al. 1974). Toxins may also play a role in increasing the effectiveness of attacks against corallivorous predators besides physical aggression (Lassig 1981). Toxicity may also act as a deterrence of interspecific competitors (Lassig 1981) and as a possible protection against pathogens (e.g. parasites, bacteria) (Munday et al. 2003).

Skin toxins in *Gobiodon* are assumed to be secreted by epidermal peculiar secretory glands, which surround the body except for the fins (Hashimoto et al. 1974). Usually, crinotoxins are only released in the course of physical destruction of the stratified epithelium (Lawrence and Smith 1989, Lassig 1981). Though, it is discussed whether coral gobies may be capable of actively controlling the production and secretion of mucus (and toxin) (Lassig 1981), but this has

not been investigated yet. Once released, the toxin disappears rapidly, probably due to enzymatic degradation (Hashimoto et al. 1974).

It is expected to have toxic representatives in both cryptic and exposed species, with a higher incidence of toxicity occurring in exposed species, because a higher predation pressure requires higher investment into defensive mechanisms (Bakus 1981, Proksch 1994, Schubert et al. 2003). Schubert et al. (2003) investigated species-specific toxicity levels within six *Gobiodon* species of the Great Barrier Reef via the bioassay method and found evidence that cryptic specimens are less toxic. Bioassays were firstly applied by Thomson (1964) to measure the effects of a toxin on another toxin-sensitive living organism (Bakus 1981, Lassig 1981). Further it was shown by the use of syntheticised food pellets that predators would avoid food containing *Gobiodon*-skin extracts (Schubert et al. 2003).

Usually, such feeding bioassay methods are used to investigate possible predator deterrence effects of toxic substances (Pawlik and Fenical 1992, Nagle and Paul 1998, McClintock et al. 1999, Pisut and Pawlik 2002, Mahon et al. 2003, Schubert et al. 2003). However, near-natural observations are sparse in the field of toxicology, and only a few studies have used video monitoring to gain information about predator-prey relationships and predation risk on a more realistic level (Christensen 1996, Nemeth 1997, Mahjoub et al. 2008, Staudinger et al. 2011). I decided to directly assess predator-*Gobiodon* interactions and the potential benefits gobies derive from toxicity for the first time by using experimental video analysis. I hypothesised (1) that toxicity acts as predation deterrent, suggesting coral gobies are despised if another non-toxic prey is optionally offered, and (2) that predation risk of *Gobiodon* spp. is variable and related to the species-specific life style (cryptic versus conspicuous habit and colour) (Hashimoto et al. 1974), therefore showing a predator-preference for more cryptic species. Based on the results from Schubert et al. (2003), I further hypothesised (3) that predator preferences are related to differing toxicity levels. To identify variations in toxicity of *Gobiodon* species in the Red Sea I conducted bioassay experiments similar to Schubert et al. (2003). I chose one representative of both conspicuous (*G. histrio*) and cryptic species (*Gobiodon* sp.3) for experiments. For understanding predator-prey interactions better, I also investigated the persistence of toxins in the water over time, as well as the release and recovery times of harmful doses of toxicity.

4 MATERIAL AND METHODS

4.1 Study area

This thesis was produced at the Department of Integrative Zoology at the University of Vienna. The field work was carried out between October and December 2010 at Dahab, Sinai (28°28' N, 34°30' E), southern Gulf of Aqaba, northern Red Sea, Egypt (figure 1). Experimental work was performed in the Dahab Marine Research Centre (DMRC), which was located about 1 km from the study sites.

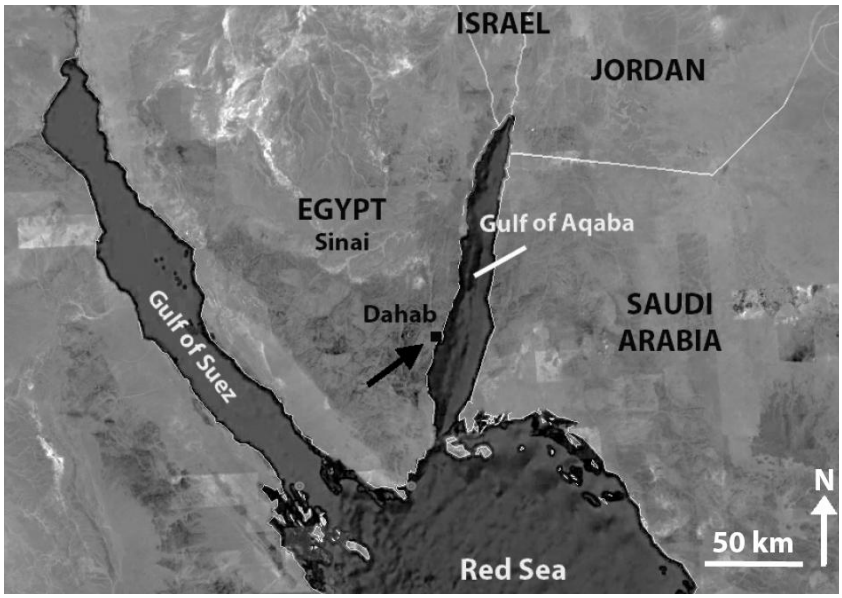

Fig. 1: Geographic location of Dahab, Sinai, Egypt. Reference: Google Maps JavaScript API, Version3: http://g.co/maps/2rt8p

Depending on habitat preferences of the different fish species required for my experiments, three sites were chosen (figure 2): predators were either collected in the „Soliman Reef" (sea grass habitat) or in the „Napoleon Reef" (relatively continuous coral cover on the reef flat), where also *G. histrio* and *G.* sp.3 were collected. Bioassay and control species (*Chromis viridis*) for all my experiments were collected in the „Lagoon" (dispersed coral patches with large sandy areas).

4.2 Experimental design for bioassay experiments

To examine variable toxicity levels among two species of *Gobiodon* from the Red Sea and to determine the time when toxicity decreases after release, I conducted bioassay studies for both *G. histrio* and *G.* sp.3.

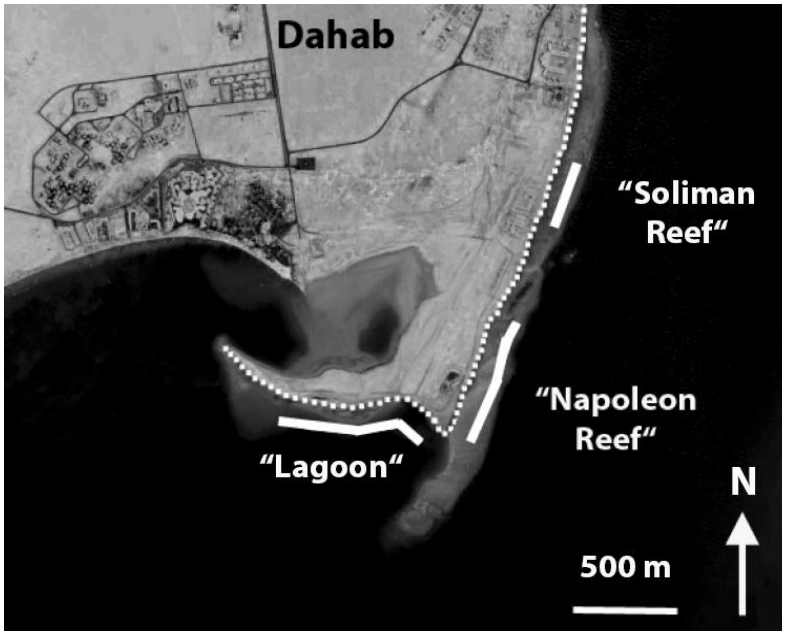

Fig. 2: Geographical location of study sites in the area of Dahab, Egypt. Dotted line indicates shore with adjacent fringing coral reefs. Thick lines approximately indicate named reefs. Reference: Google Maps JavaScript API, Version 3: http://g.co/maps/rdan3

4.2.1 Preliminary studies and fish collection

Although effects on locomoter capacity was evident in several other species tested as bioassay fish, I chose *C. viridis* (= *C. vir*) for my experiments, a common planctivorous coral-associated damselfish, because collection was easy within large schools of uniformly sized individuals with similar post-settlement history and toxin-related symptoms appeared clearly fast. This assured their sensibility to *Gobiodon* skin secretions, which was in particular important because *C. viridis* potentially shares habitat with *Gobiodon* species (Mohamed Negm, personal communication).

A total of 10 *G. histrio* (= *G. his*) and nine *G.* sp.3 and 228 *C. viridis* were caught within 8 days (total length (TL) in cm: *G. histrio* = 4.5 ± 0.3, *G.* sp.3 = 4.5 ± 0.3, *C. viridis* = 3.7 ± 0.5) by anaesthetisation using clove oil (10 ml in 40 ml of 95% ethanol, diluted with 200 ml seawater), which is efficient even in low concentrations without causing permanent damage to the fish narcotised (Munday and Wilson 1997, Hajek et al. 2006). Specimens of *C. viridis* were kept in one large tank supplied with stones for shelter for acclimatisation, whereas gobies were kept individually and separately.

4.2.2 Species-specific toxicity using bioassay

For each trial one *Gobiodon* was stressed for 20 seconds by gently rubbing the fish inside a plastic bag containing 10 ml of seawater, yielding a foamy, milky, mucous secretion (Schubert et al. 2003). The plastic bag was rinsed with 90 ml of seawater and added up to a total of 600 ml. The seawater used for dilution was taken from the tank where *C. viridis* were maintained overnight to exclude undesirable side-effects. The mixture was shaken for equal dilution and then poured into two 300 ml examination buckets. One *C. viridis* was put into each of the two buckets by not later than 1 minute after the donator goby was rubbed. Using two individuals for each run instead of one (Schubert et al. 2003) enabled a slight correction of potential differences in sensitivity between bioassay specimens. Thereby, a total of 38 *C. viridis* (2 for each goby) were used for this experiment. Time to loss of equilibrium was measured using a stop watch and behaviour of individuals was noted. Loss of equilibrium time, referred to as time until a bioassay specimen shows loss of locomotory abilities and balance in response to a chemical, is a standardized method (Munday and Wilson 1997, Schubert et al. 2003, Hajek et al. 2006) to generate an estimate of the toxin's effect. A control specimen of *C. viridis*, which was kept in 300 ml of pure seawater and changed with each pair of experimental fish, assured good general condition within the setup and excluded side-effects. After loss of equilibrium, fish were weighed, total body length was measured and condition factors (Cf) were calculated as Cf = 100*weight[g]*length^{-3}[cm]. Loss of equilibrium time of each trial was calculated as the mean loss of equilibrium time of both *C. viridis* used in this trial. *E. fasciatus* (n = 1), *G. histrio* and *G.* sp.3 (n = 2) were also tested as bioassay fishes, using the same method as described above, except for using 1800 ml and skin secretions of 6 *G. histrio* (yielding the approximately same concentration) for *E. fasciatus*.

4.2.3 Decrease of toxicity over time using repeated bioassay

To investigate species-specific declines of toxicity over time, I repeated bioassays. Therefore, I continued using the same two 300 ml mucus-seawater-solution as in the bioassay tests above. This means that each goby (10 replicate *G. histrio* and 9 replicate *G*. sp.3, respectively) was rubbed only once at the very beginning of the first bioassay test. Afterwards, loss of equilibrium time was analysed at different intervals (10, 30, 60, 120 and 240 minutes) employing two other specimens of *C. viridis* and one control at each interval. The solution was stirred one minute before each trial started, because a homogenous solution was required for comparable results. If no effect on the bioassay species was apparent, the experiment was terminated after 30 minutes.

During the whole procedure (240 min) the water was not aerated, because preliminary experiments have shown that *C. viridis* survives in 300 ml of seawater without aeration for more than 24 hours.

4.2.4 Recovery of skin toxins

The time-span of toxin recovery of *G. histrio* after previous loss of the harmful dose of toxicity was measured. A total of 21 specimens were collected in the field as described above. Each individual was rubbed for a mean time of 124 (± 20.8 S.D.) seconds in plastic bags containing 10 ml seawater each. Bags were changed every 30 seconds until secretion of toxins stopped, i.e. no milky liquid visible anymore. To assure that toxicity was eliminated, bioassay tests as described above were performed by employing the content of the last plastic bag. Subsequently, gobies were separately kept in aerated 500 ml aquariums without feeding. Only the water was changed once a day. After different time-spans (6, 12, 24, 36, 48, 120 hours) bioassay experiments were repeated to determine the required amount of time for recovery of toxicity (the number of *G. histrio* at each interval was between 3 and 5).

4.3 Experimental design for predator preferences using video-monitoring

4.3.1 Identification of *Gobiodon*-predators and fish collection

For identification of potential predators of *Gobiodon* in the field narcotised *Gobiodon* specimens were offered to different predator species: *S. cyanostigma*, *Gymnothorax griseus* (Lacepède 1803), *Thalassoma klunzingeri* (1871, Klunzinger), and *E. fasciatus* (Forsskål, 1775). Positive results (i.e. gobies were attacked and eaten when narcotised) were shown by *T. klunzingeri*, a small-gaped part-time resident, and *E. fasciatus*, a large-gaped full-time resident predator. While *T. klunzingeri* turned out to be unsuitable for aquarium experiments due to its high swimming activity, *E. fasciatus*, which is usually closely associated with the substratum, showed less activity and a much less nervous behaviour in captivity (Randall and Ben-Tuvia 1983). It was therefore chosen for the subsequent aquarium experiments. For the non-toxic control species I chose *C. viridis* that represents an important prey species for many reef-based predators (Leis and Carson-Ewart 2002, Lecchini et al. 2007). Other than *Gobiodon*, *C. viridis* exhibits full-body squamation and strong fin spines.

In total 18 *E. fasciatus* were caught within 10 days (total length (TL): mean ± S.D. = 16.3 ± 2.8 cm), each within three hours in the morning three days before recordings started using a handnet with a prismatically bended wire mesh (diameter of opening: ~ 40 cm) fixed to a PVC

pipe of 1m length. Predators were attracted by canned fish. All 54 prey specimens, 18 specimens per species (TL ± S.D.: *G. histrio* = 3.9 ± 0.5 cm; *G.* sp.3 = 4.0 ± 0.5; *C. viridis* = 3.9 ± 0.8 cm), were collected one day before video recordings started by anaesthetisation using clove oil solution (10 ml clove oil in 40 ml 95% ethanol and diluted with 200 ml seawater).

4.3.2 Aquarium setup

Before video-recording started, *E. fasciatus* were starved for 2.5 days to assure fish were motivated to feed. For the first 1.5 days they were kept in outdoor aquariums with continuous water flow and then transported to indoor aquariums to allow adaptation to indoor light and temperature conditions one day before recordings started. All aquariums were aerated and equipped with separation walls. Total body length was measured during transportation in a box (using nets caused much greater excitement). In the evening predators were relocated from the acclimatisation tank into the aquariums provided for video recordings (figure 3), one predator in each container. Containers (each of a volume of 20 litres) were installed in two tanks (A1 and A2), of 130 litres capacity each, and were equipped with white bottoms for good contrast in video recordings. Above each container, cameras were fixed to a base frame to allow for a permanent set. The third tank (A3) provided additional water supply and assured consistent water quality through filtering and aeration (volume: 200 litres).

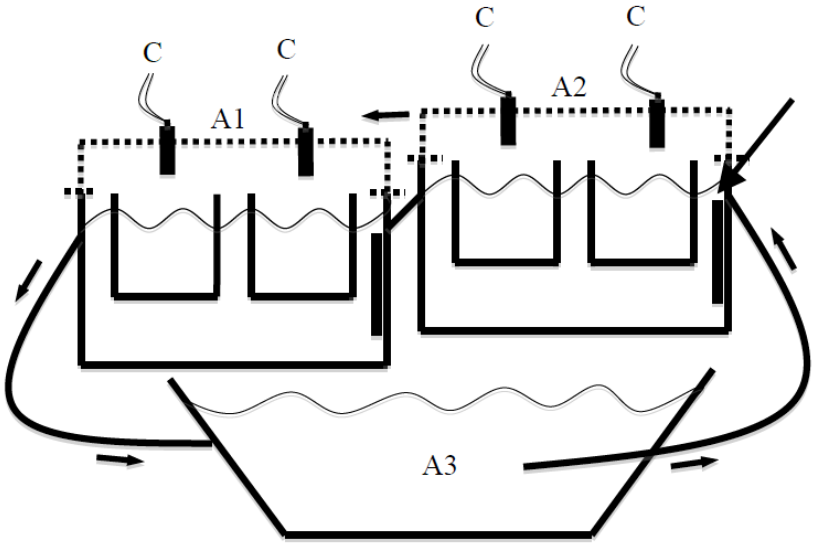

Fig. 3: Aquarium setup. A1 and A2 indicate tanks for video analysis containing two containers with video cameras above (C = Cameras). Tank A3 was equipped with a circulator pump for continuous water flow, filter pump and aeration. Dotted lines indicate base frame for holding cameras in place, small arrows represent the direction of water flow and the large arrow points out the downpipe assuring even water surface for video recording.

Preliminary experiments revealed that the stress level of predators was kept low by allowing for acclimatisation to environmental conditions over night. In the morning of day 4 after video recordings were started between 8 and 9 am using a Neostar (MPEG 4) digital video recorder. One prey specimen of each species (*G. histrio, G.* sp.3 and *C. viridis*) was introduced to each *E. fasciatus* after size measurements and weighing. To minimize predator-prey size differences, smaller prey specimens were attributed to smaller predators and larger prey to larger predators. Recordings yielded a total of about 9.5 hours of video footage for each predator. Predators were then weighed and kept for observation of their health condition overnight. All specimens were released back to the reef in the following morning.

4.3.3 Video analysis

At the University of Vienna video analysis was conducted twice to exclude variations due to learning processes in the course of this survey. Videos were analysed using "BACKUP Player" included in CMS- (Central Management Service) Software. The behavioural categories of predators and preys which were analysed are listed in table 1 and 2.

The duration until an event occurred was recorded, calculated as the time from when prey specimens entered the water to a predator's action - determined by prey crypticity and predator sensory capacities (Christensen 1996). Also noted were the number (one, two or all three prey species) (Christensen 1996) and condition (alive or dead) of predator-affected prey species as well as distances between all fishes at the moment immediately before an event occurred (i.e., a maximum of three predator-prey-, and three prey-prey-distances) (Mahjoub et al. 2008, Nemeth 1997). Distances were measured from each fish's snout tip. Although distances measured in 2D did not align parallel to the camera view, this was neglected since distance was only used as an approximate variable.

Tab. 1: Categories used for quantification of predator-prey interactions. ''x" indicates categories that may include a preceding approach (recorded only if there was a short time gap between approach and a following behaviour). *Italics* indicate categories accompanied by strike capture.

Category	**Abbr.**	**Characterisation**	**Reference**
approach	A	definite active movement of a predator towards the direction of at least one prey specimen; sometimes followed by strike capture	Mahjoub et al. (2008)
strike capture [x]	SC	sometimes preceding to approach; sudden jump of the predator towards the prey to engulf it successfully; possibly followed by ingestion/consumption (whole prey)	Mahjoub et al. (2008)
consumption		additionally recorded as "fish dead"	Christensen (1996) Mahjoub et al. (2008) Staudinger et al. (2011)
manipulation time	MT	handling time of a prey in the predator's mouth	
strike capture partially [x] (rarely)	SCP	prey was not captured completely	
strike failure [x]	SF	failed targeted attack in which predator initiated an aggressive movement towards prey and opened its mouth, but either prey fled or attack was abandoned	Staudinger et al. (2011) Mahjoub et al. (2008)
total regurgitation	TR	expel of a prey's total body (both alive or dead)	
partial regurgitation	PR	body part (fins, head or tail) was expelled and visible outside the predator's mouth (followed by total TR or ingestion)	

Tab. 2: Categories used for quantification of predator (first three) and prey (last three) behaviour.

Category	Characterisation	Reference
approach/strike (A/S) velocity	fast or slow	Christensen (1996) Mahjoub et al. (2008)
multistaged assembly of approaches and strikes	approach/strike was not realised at once, but serially	
touch	physical contact with prey (predators frequently touch prey with their nose)	
initial movement (before event)	corresponding to the predator's attention (active or motionless prey)	
anti-predator responses	attempts to escape from a predator's approach or strike (fleeing or even jumping out of the water); sometimes followed by strike capture	Christensen (1996) Staudinger et al. (2011)
behaviour after regurgitation	prey moving or motionless	

4.4 Statistical analysis

4.4.1 Bioassay experiments

For analysis of species-specific loss of equilibrium times Mann-Whitney-U-test was used. For analysis of toxicity decrease, I employed a one-way-ANCOVA (analysis of covariance), which tests the equality of means for several univariate groups (loss of equilibrium times for all intervals). ANOVA and t-tests were used to test for homogenous means of weight and condition factor data for all *C. viridis* used in all intervals, because samples were normally distributed and had similar variances.

For the predator preference experiments I used Chi-square-tests (Chi^2) for direct comparisons between *C. viridis* and both gobies regarding to pre-capture events (move, nearest prey) or for comparisons among observed and expected values of preys eaten. Mann-Whitney-U and Kruskal-Wallis tests were employed for pair-wise comparisons or across all three groups regarding to distances, preferences among single or grouped prey and regurgitation rates. All analyses were carried out with PAST, ver. 2.12 (Hammer et al. 2001).

4.4.2 Predator preferences experiments

To estimate survival probabilities, a survival analysis was carried out, within which the time to an event is modeled. More advanced analyses are used to compare between two or more groups to estimate the effects of certain variables (covariates) on the event. During data analysis attention must be paid to censored data, e.g. observations, where the event to be modeled (usually death) did not occur during the observation period. Ignoring these data would bias calculations towards low survival rates, as long-term survivors would be eliminated from the data set. The development of statistical methods such as Kaplan-Meier-estimator or Cox-regression has helped dealing with this kind of data (Glantz 1998).

Within the present study the Kaplan-Meier-estimator was used for graphic representation of the survival rates, to calculate mean and median survival times. It is a non-parametric method to calculate the survival function for a specific group of observations. Whenever a relevant event occurs (in this study: a fish was eaten or dead) the probability of one individual of the given population to survive past time *t* is:

$$S(t) = (ni - di) / ni$$

where *ni* = the individuals at risk at time *t* (e.g. survivor minus number of censored observations since the last event) and *di* = number of individuals, for which the event occurred at time *t*. In this analysis the survival probability does not change between events. The Kaplan-Meier-estimator is the product of all survival probabilities observed.

The Cox-regression or proportional hazard model (Hill and Lewicki 2007), which is commonly used in medical studies, was used for estimating the influence of certain covariates on survival times (Cox 1972, Ziegler et al. 2007, Staudinger and Juanes 2010). This model assumes that the hazard function for an individual depends on the values of the covariates and the value of the baseline hazard. Within the predator preferences experiment, covariates were regurgitation, manipulation, type of species and number of species alive, condition factors and weights of predators and preys. Covariates employed in the Cox-regression must not be correlated with each other and were previously checked for intercorrelation. Since there was a significant negative correlation between total length of both *Gobiodon* species and their condition factor (*G. histrio*: $R^2 = 0.8$, $p < 0.001$; *G.*sp.3: $R^2 = 0.2$, $p = 0.1$) and a correlation between total length and weight (*G. histrio*: $R^2 = 0.84$, $p < 0.001$; *G.* sp.3: $R^2 = 0.96$, $p < 0.001$), I employed condition factor and weight only.

The model may be written as:

$$h\{(t), (z_1, z_2, ..., z_m)\} = h_0(t)*\exp(b_1*z_1 + ... + b_m*z_m)$$

where *h(t,...)* represents the resultant hazard (= risk of an individual to die), given the values of the *m* covariates for the respective case (z_1, z_2, ..., z_m) and the respective survival time (*t*). The term $h_0(t)$ indicates the baseline hazard.

Both Kaplan-Meier-estimator and Cox-regression were carried out in IBM SPSS Statistics 20 for Windows 7.

5 RESULTS

5.1 Bioassay

5.1.1 Species-specific toxicity using bioassay

Compared to control specimens of *C. viridis*, who kept well during all experiments, *C. viridis* exhibited symptoms of initial irritability and motor excitation, as well as loss of equilibrium in response to skin secretions of both *G. histrio* and *G.* sp.3 during all 38 trails proceeded [(9 + 10)*2]. The time to loss of equilibrium of *C. viridis* one minute after gobies were rubbed constitutes a median of 75 sec (55.5, 82; lower quartile, upper quartile) for *G. histrio* and 105 sec (80, 131.5) for *G.* sp.3, indicating a significance difference between both species (Mann-Whitney-U-test: U = 79, p = 0.003). Weights of experimentally used *C. viridis* did not show significant differences between trials with both goby species (mean ± S.D. for *G. histrio* trials = 1.0 ± 0.4 grams (g) and for *G.* sp.3 trials = 1.1 ± 0.4 g , Mann-Whitney-U-test: U = 165.5, p = 0.6). The condition factor of all *C. viridis* also showed no significant differences (mean ± S.D. = 1.8 ± 0.2 for *G. histrio* trials and 1.8 ± 0.3 for *G.* sp.3 trials, Mann-Whitney-U-test: U = 169.5, p = 0.7). This confirms homogeneity of individuals between the two experimental series. Contrary to *G. histrio* and *G.* sp.3, which did not show any signs of stress after exposure to toxins of both *G. histrio* and *G.* sp.3, *E. fasciatus* (n = 1) exhibited loss of equilibrium after 40 minutes.

5.1.2 Decrease of toxicity over time using repeated bioassay

In the test intervals up to 30 minutes all *C. viridis* lost equilibrium and out of the 38 *C. viridis* tested after 60 minutes 37 exhibited loss of equilibrium. In contrast, at longer intervals (120 and 240 minutes) a greater number of *C. viridis* did not exhibit loss of equilibrium within the observation time of 30 minutes (at 120 minutes *G. histrio*: 15%, *G.* sp.3: 11% and at 240 min *G. histrio*: 70%, *G.* sp.3: 50% of all *C. viridis*). Due to the respective lack of loss of equilibrium data at 120 and 240 minutes intervals, only test intervals of up to 60 minutes were further analysed (figure 4). The toxicity-level of *G. histrio* was significantly higher than that of *G.* sp.3 at the 1 minute-interval (Mann-Whitney-U-test for log data: U = 17, p = 0.02) and close to significant difference at the 10 minutes–interval (Mann-Whitney-U-test for log data: U = 21, p = 0.055), but not significant in the longer intervals (figure 4a). ANCOVA (figure 4b) also revealed a significantly higher basic toxicity of *G. histrio* (group mean difference: p = 0.002). A decrease of toxicity over time is obvious and equal in both species (homogeneity of slopes: F = 0.26, p = 0.61).

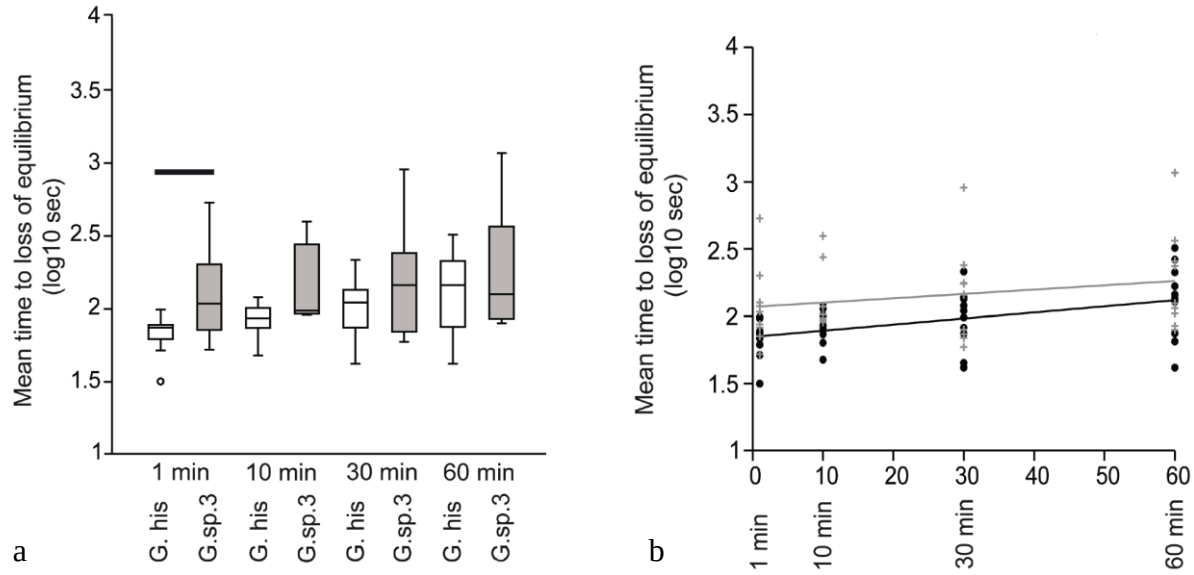

Fig. 4: Loss of equilibrium times of *Chromis viridis* when exposed to skin toxins of *Gobiodon histrio* (white) and *G.* sp.3 (grey). a: mean time to loss of equilibrium in log10 of seconds. Boxes represent quartiles, central lines the medians, and whiskers the range of data. Horizontal line indicates significant difference ($p < 0.05$; Mann-Whitney-U-test). b: One-way-ANCOVA for *G. histrio* (black) and *G.* sp.3 (grey).

Chromis viridis specimens did not differ significantly between intervals, neither in their weight (ANOVA: $p > 0.07$ for all pairwise comparisons: *G. histrio* trials = 1.1 ± 0.4 g, *G.* sp.3 trials = 1.2 ± 0.4 g) nor in their condition factors (ANOVA: $p > 0.05$ for all pairwise comparisons (*G. histrio* trials = 1.8 ± 02, *G.* sp.3 trials = 1.9 ± 0.3). In the case of G. histrio, toxicity was related to fish size, exhibited by a significantly positive correlation between loss of equilibrium time of *C. viridis* and the length of the 10 G. histrio toxin donators in the most relevant test intervals ($R^2 > 0.41$, $p < 0.01$ for the intervals 1 to 60 min). No such correlation was found for *G.* sp.3.

5.1.3 Recovery of toxins

Gobiodon histrio specimens which were deprived of their entire epidermal skin toxins, were not able to rebuilt toxicity before 48 hours (n gobies per interval = 3) (figure 5). The first apparent effect on the equilibrium of *C. viridis* bioassay specimens was shown after 48 hours (n = 5) with a median loss of equilibrium time of 341 seconds (225, 341; lower, upper quartile) and, after 120 hours (n = 4), 389 seconds (258, 638).

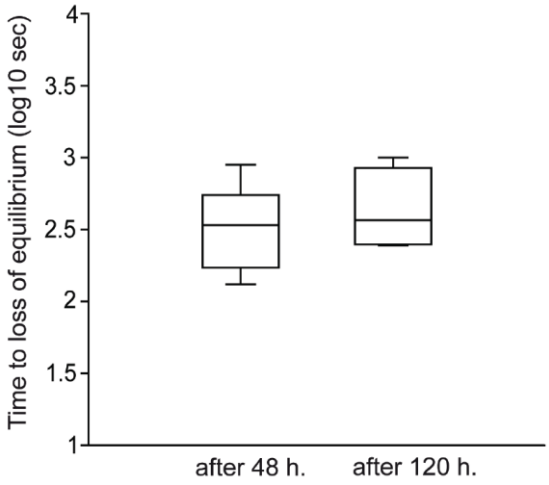

Fig. 5: Loss of equilibrium time of *C. viridis*-bioassay specimens after 48 (n = 5) and 120 (n = 4) hours of skin toxin recovery of *G. histrio* (in log10 of seconds).

5.2 Predator preferences using video-monitoring

Out of a total of 18 predators, 16 predators consumed at least one prey, yielding a total of 33 consumed prey specimens. Fourteen predators consumed *C. viridis*, eleven predators consumed *G. histrio* and eight predators consumed *G.* sp.3. A total of 1139 events were recorded during 165 hours of video, whereas 58.5 % of all events were approaches, 21.3 % were strikes and 20.2 % represented other behavioural categories.

5.2.1 Pre-capture events

The sixteen active predators performed a total of 668 approaches during recordings. Each predator approached at least one prey species, and most of them approached more than one (figure 6a). Only 14 % of all approaches were followed by a strike. Though, by far most approaches (86 %) did not result in a strike (figure 6b). Only *Gobiodon* species were touched by predators (with their snout), which occurred most often during approaches (table 3). Escaping from a predator, referred to as anti-predator response, was exhibited by all three species, although more *C. viridis* fled when experiencing an approach or a subsequent strike capture. The four strike failures towards *Gobiodon*, which did not exhibit a response, indicate that the strikes were aborted by predators and not due to escape behaviour of the prey. Jumping out of the water was only occasionally used by any of the three species.

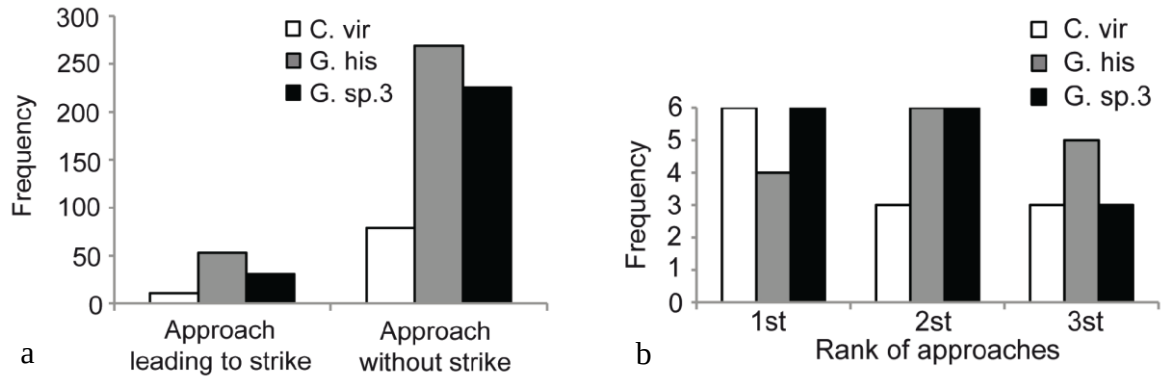

Fig. 6: Analysis of approaches of *E. fasciatus* towards three different prey species. a: Frequency of approaches arranged in the sequence by which species were approached by predators within trials (number of predators = 16). Repeated approaches of the same species are neglected. Ranks are slightly biased by consumptions without approaches. b: Approaches leading to a strike (left) or not (right).

Tab. 3: Analysis of predator and prey behaviour.

		touch		**anti-predator response**		**jump**	
Species		**0 (%)**	**≥ 1 (%)**	**0 (%)**	**1 (%)**	**0 (%)**	**≥ 1 (%)**
C. vir	approach	90 (100)	0	15 (17)	75 (83)	80 (89)	10 (11)
	strike capt.	16 (100)	0	6 (38)	10 (62)	14 (88)	2 (12)
	strike fail.	6 (100)	0	0	6 (1)	3 (50)	3 (50)
G. his	approach	222 (77)	66 (23)	170 (59)	118 (41)	252 (88)	36 (12)
	strike capt.	67 (84)	13 (16)	36 (45)	44 (55)	77 (96)	3 (4)
	strike fail.	33 (97)	1 (3)	2 (6)	32 (94)	27 (79)	7 (21)
G. sp.3	approach	199 (78)	56 (22)	192 (75)	63 (25)	237 (93)	18 (7)
	strike capt.	52 (95)	3 (5)	29 (53)	26 (47)	51 (93)	4 (7)
	strike fail.	22 (92)	2 (8)	2 (8)	22 (92)	20 (83)	4 (17)

Touch: predator touching prey once or more often (≥ 1) or not (0); anti-predator responses of prey (1) versus no anti-predator responses (0); one or more jumps of prey out of the water (≥ 1) versus no jump (0); (n of predators: approaches = 16; strike captures = 17; strike failures =14).

Chromis viridis moved significantly more often than both coral gobies before approaches (percentage of "move" and "no move" for *C. viridis* versus pooled gobies: $Chi^2 = 184$, df = 1, $p < 0.001$), suggesting *C. viridis* is the most active prey species. In contrast, strikes were performed regardless of the movement of an aimed prey. Multi-staged approaches are generally used by predators to overcome long distances. Because *C. viridis* was usually approached from greater distance, more multi-stage approaches occurred. The velocity performed by predators towards prey generally appeared to be rather low. In most cases, approached or strike captured prey was closest to a predator (= nearest neighbour). However, approaches were – compared to strike captures – less often performed towards the nearest neighbour, in particular for *C. viridis.* The latter species was more frequently approached when not being the nearest neighbour compared to both gobies (*C. viridis* versus mean of pooled gobies: proportion of approaches towards nearest versus not nearest neighbour: $Chi^2 = 28.6$; df = 1, $p < 0.001$) (table 4), and was also approached from a greater total distance (figure 7). All three species were usually the nearest neighbour when experiencing a strike capture (*C. viridis* versus mean of pooled gobies: proportion of strike captures towards nearest versus not nearest neighbour: $Chi^2 = 0.7$; df = 1, $p < 0.03$). Generally, approach distances for all species are significantly longer than strike capture distances (Mann-Whitney-U-test for *C. viridis*: $U = 223$, $p < 0.001$, for *G. histrio:* $U = 3834$, $p < 0.001$, and for *G.* sp.3: $U = 2468$, $p < 0.001$) (figure 7).

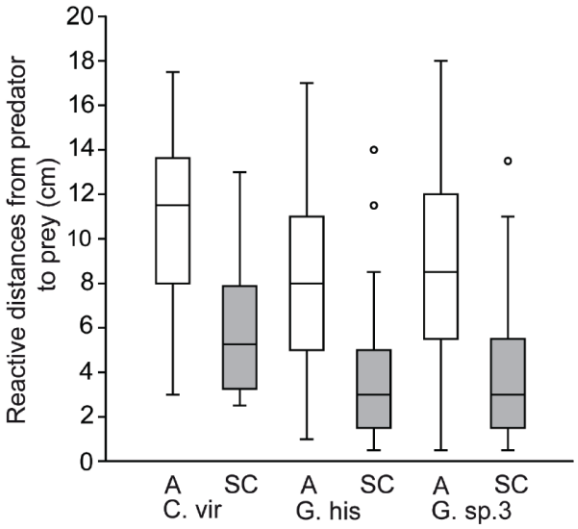

Fig. 7: Reactive distances of predators to each prey species before an approach (A, white boxplots) or strike capture (SC, grey boxplots).

Distances are significantly longer for *C. viridis* than for each of the two gobies before approaches (*C. viridis* versus both gobies: Mann-Whitney-U-test: $p < 0.001$, Bonferroni corrected) and before strike captures (*C. viridis* versus both gobies: Mann-Whitney-U-test: $p < 0.03$, Bonferroni corrected), whereas there was no difference between the two goby species (Mann-Whitney-U-test: $p > 0.6$ for approaches and strike captures).

Tab. 4: Analysis of predator and prey behaviour.

species	category	nearest NB (%)	move		multi-stage A/S		A/S velocity	
			0 (%)	1 (%)	0 (%)	1 (%)	slow(%)	fast (%)
C. vir	approach	30.0	16 (18)	74 (82)	34 (38)	56 (62)	77 (86)	13 (14)
	strike capt.	78.3	8 (50)	8 (50)	11 (69)	5 (31)	1 (6)	15 (94)
	strike fail.		2 (33)	4 (67)	5 (83)	1 (17)	0	6 (100)
G. his	approach	66.6	269 (93)	19 (7)	139 (48)	149 (52)	242 (84)	46 (16)
	strike capt.	89.2	43 (54)	37 (46)	50 (63)	30 (37)	27 (34)	53 (66)
	strike fail.		17 (50)	17 (50)	26 (77)	8 (23)	6 (18)	28 (83)
G. sp.3	approach	69.1	244 (96)	11 (4)	122 (48)	133 (52)	219 (86)	36 (14)
	strike capt.	76.7	28 (51)	27 (49)	34 (62)	21 (38)	18 (33)	37 (67)
	strike fail.		9 (37)	15 (63)	18 (75)	6 (25)	3 (13)	21 (87)

Nearest neighbour: attacked prey is closest to predator; move: at time shortly before an event prey actively moves (1) or does not move (0); multi-stage A/S: either predator performs approach (A) or strike (S) at once (0) or in several steps (1); A/S velocity: A predator's slow or fast speed during an approach (A) or strike (S). Number of predators: approaches = 16; strike captures = 17; strike failures =14.

More approaches were performed towards grouped prey specimens compared to single individuals, although not significant (Mann-Whitney-U-test: $U = 0$, $p = 0.08$), suggesting prey-groups are appealing for predators. In contrast, preferably single specimens were captured, although not significant (Mann-Whitney-U-test: $U = 2$, $p = 0.3$), even when more than one prey species was alive. Strike failures seemed not to be triggered by the number of species affected by a strike, because predators of striking both single and groups of preys experienced failures (figure 8).

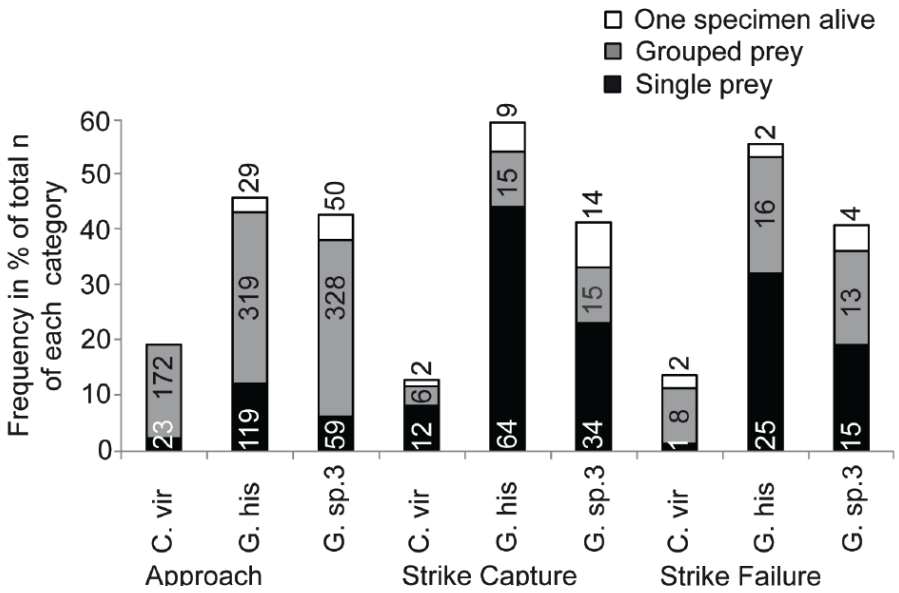

Fig. 8: Number of species affected by predators' approaches or strikes with respect to number of species alive at that moment. One specimen alive: 1 species alive and affected*; Grouped prey:* 2 or 3 prey species alive and affected; Single prey: 2 or 3 preys alive, 1 prey affected (total n of predators: A = 16, SC = 16, SF = 12). Each frequency for each species is counted (e.g. if an approach is performed towards all three species, it is counted for each species, thereby enhancing the total value of approaches by doubling or three-folding some total frequencies). Numbers in or above bars indicate total number of cases.

5.2.2 Strikes and post-capture events

All analyses of strike captures and regurgitations (figures 8, 9 and 11) only include strike captures and regurgitations of prey fishes that were still alive, because, although happening rarely, some prey fishes died during manipulation by the predator but were still repeatedly regurgitated and captured.

Within all three prey species regurgitations after strike capture occurred, though regurgitation rates varied greatly among species. All captured specimens of *C. viridis* were finally consumed, regardless of regurgitations. Survivors of captured specimens only occurred among *G. histrio* and *G.* sp.3, suggesting an interest of predators in all prey species but with different rates of final consumption. As clearly demonstrated (figure 9, table 5) the number of *C. viridis* immediately eaten (= without regurgitation) is significantly higher than in gobies (12 of 18 *C. viridis* and 6 of 36 gobies immediately eaten (= observed) and 14 of 18 *C. viridis* and 19 of 36 gobies (= expected): calculated with proportional ratio for observed and expected versus total individuals offered: $Chi^2 = 54.4$, $df = 2$, $p < 0.001$).

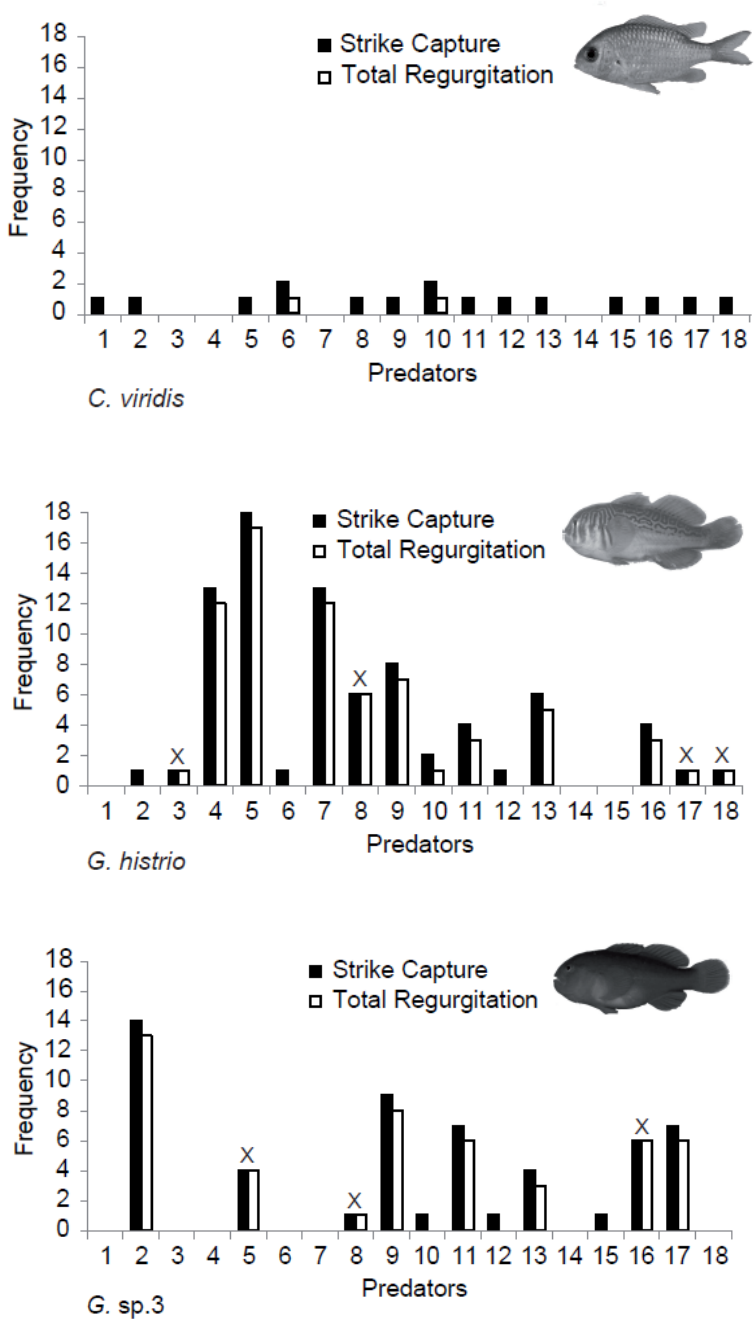

Fig. 9: Strike capture and total regurgitations for *C. viridis*, *G. histrio*, and *G.* sp.3 for all 18 predators. "X" indicates specimens, which survived the experiment despite single or multiple captures. In all other cases prey species were consumed during the experiment: either immediately (= one strike capture, which is not neccessarily the first strike performed by a predator, without subsequent regurgitation), or after preceding regurgitations.

Because most strike captures towards gobies resulted in regurgitation instead of immediate consumption, the number of species affected by strike capture (with or without subsequent regurgitation) and the rank of consumption differ, whereas only in the latter clear preferences

were apparent (figure 10a and 10b). Of all 16 preys consumed (figure 10c) first, 10 specimens (62.5%) were *C. viridis*, 4 specimens (25 %) were *G. histrio* and 2 specimens (12.5%) were *G.* sp.3 (Chi^2-test on proportional data (62.5% for *C. viridis* and 37.5% for both gobies observed versus 33.3% and 66.6% expected): $Chi^2 = 38.3$, df = 1, $p < 0.001$).

Tab. 5: Strike captures and consumptions of all three prey species from 16 predator preference experiments.

species	total n eaten (% of 16)	immediately eaten (% of 16)	survivors (% of 16)	alive: SC with TR (% of all SC)	dead: SC with TR (% of all SC)
C. vir	14 (88)	12 (75)	0	2 (1)	10 (6)
G. his	11 (69)	3 (19)	4 (25)	77 (44)	16 (9)
G. sp.3	8 (50)	3 (19)	3 (19)	52 (30)	0

Total n eaten: total number of specimens eaten during experiments; immediately eaten: eaten without regurgitation (not necessarily at first); survivors: captured and regurgitated prey without final consumption; alive: SC with TR: strike captures of live specimens; dead: SC with TR: strike captures of specimens that had died from predator manipulation; (total number of strike captures = 175).

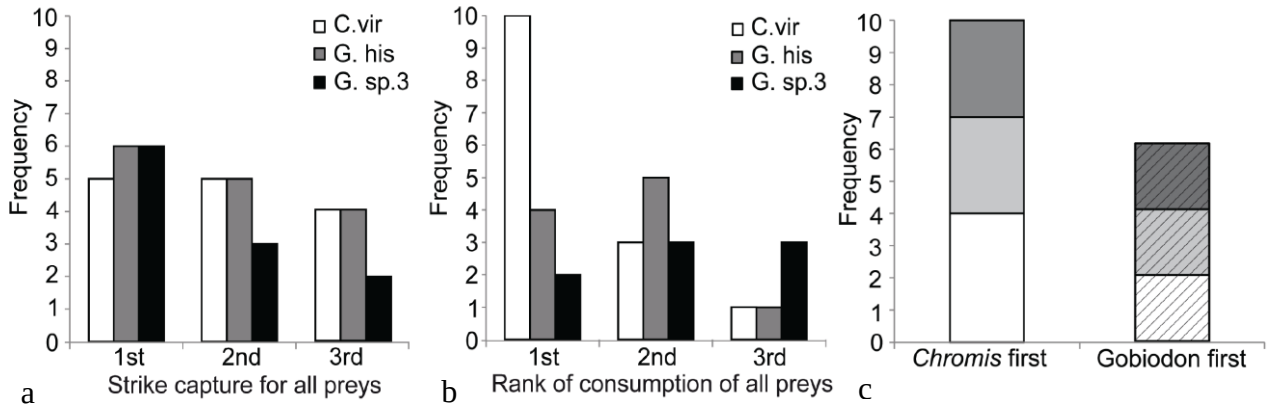

Fig. 10: Analysis of strike captures of *E. fasciatus* towards 3 different prey species. a: Frequency of strike captures of all preys regardless of subsequent consumption, arranged in the sequence by which species were captured for their first time. The frequency of second and third strike captures may be influenced by prey eaten at the first consumption (n predators = 17). b: Rank of strike captures with subsequent consumption (n predators =16). c: Prey selection depending on first prey eaten (left: *C. viridis*, right: goby); white: all three prey fishes consumed; light grey: one *C. viridis* and one goby consumed; dark grey: either a *C. viridis* or a goby consumed.

Furthermore, out of 16 predators, five consumed only one prey in total, five consumed two prey specimens (*C. viridis* or goby first) and six predators consumed all three preys. Of all predators consuming *C. viridis* at first, 70% consumed also at least one goby and 66% of predators consuming a goby first consumed also a *C. viridis* and/or a second goby. It never occurred that a predator consumed 2 gobies only.

In contrast to the high frequencies of strike captures, strike failures occured rarely and the frequency per predator had a mean ± S.D. of 1.2 ± 0.5 for *C. viridis* (n = 5), 3.4 ± 2.6 for *G. histrio* (n = 10) and 2.4 ± 1.4 for *G.* sp.3 (n = 10).

Of all preys finally consumed regurgitation frequency (analysed for totally regurgitated prey only) was the highest for *G. histrio*, although not significantly different from *G.* sp.3 (Mann-Whitney-U-test: p = 1, Bonferroni corrected) (figure 11) and was significantly higher for both gobies compared to *C. viridis* (Mann-Whitney-U-test: *G. histrio* p = 0.004, *G. sp.3*: p = 0.02, Bonferroni corrected), because they were frequently captured but regurgitated after very short manipulation times. *Chromis viridis* experienced a regurgitation frequency close to zero (the only regurgitated specimens were dead and regurgitated simultaneously with a previously captured goby). The maximum number of regurgitations for prey specimens not consumed finally was much lower and only occurred for *G. histrio* and *G.* sp.3, with the latter having a slightly higher regurgitation frequency (figure 11).

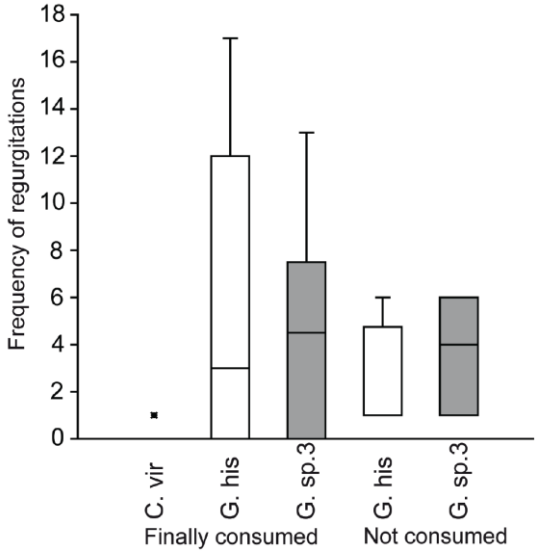

Fig. 11: Frequency of regurgitations for all prey species, shown separately for prey finally consumed and not consumed.

Manipulation time was strikingly increased by some predators after a certain cumulativemanipulation time of *G. histrio*: (n = 5, mean of 100 sec) and *G*. sp.3 (n = 5, 52 sec), accompanied by a decrease in regurgitation frequency, where upon gobies were finally consumed. Manipulation time per capture did not differ remarkably among species (table 6), whereas manipulation times at the first strike capture were much lower and cumulative manipulation times until death were much higher in the gobies. In contrast to gobies, manipulation time of *C. viridis* remained relatively constant between first strike, per capture and until death (table 6), due to the great amount of immediate consumptions of *C. viridis*.

Tab. 6: Manipulation times of all three prey species from 16 predator preference experiments.

Species	**Median MT at first SC (lq, uq)**	**Median MT/SC (lq, uq)**	**Median MT of prey alive (lq, uq)**
C. vir	25 (7, 32)	26 (7, 68)	26 (7, 68)
G. his	7 (4, 28)	24 (14, 61)	89 (45, 212)
G. sp.3	4 (1, 45)	16 (6, 63)	107 (9, 174)

MT: Manipulation time (in a predator's mouth) in seconds; MT at first SC: MT at first strike capture of a prey species (in sec); MT/SC: MT per strike capture; MT of prey alive: cumulative MT until a prey's death. lq = lower quartile; uq = upper quartile.

The cumulative survival rate for *C. viridis* was much lower than in both *Gobiodon* species, but almost equal for both coral gobies (figure 12). The Cox-regression, which was used for estimating the influence on certain covariates on survival times, revealed that there were no significant influences of covariates on the survival of *C. viridis* ($p > 0.1$ for the covariates regurgitation rate per hour, mean manipulation time, condition factor of prey and predator). Overall, the Cox-regression for both *Gobiodon* species (table 7) was highly significant ($p < 0.001$), indicating important effects of covariates on the probability of survival. A positive coefficient B predicts a greater risk of being eaten. Therefore, in this analysis, higher regurgitation rates affect the survival of both gobies negatively, and a high condition factor increases predation risk.

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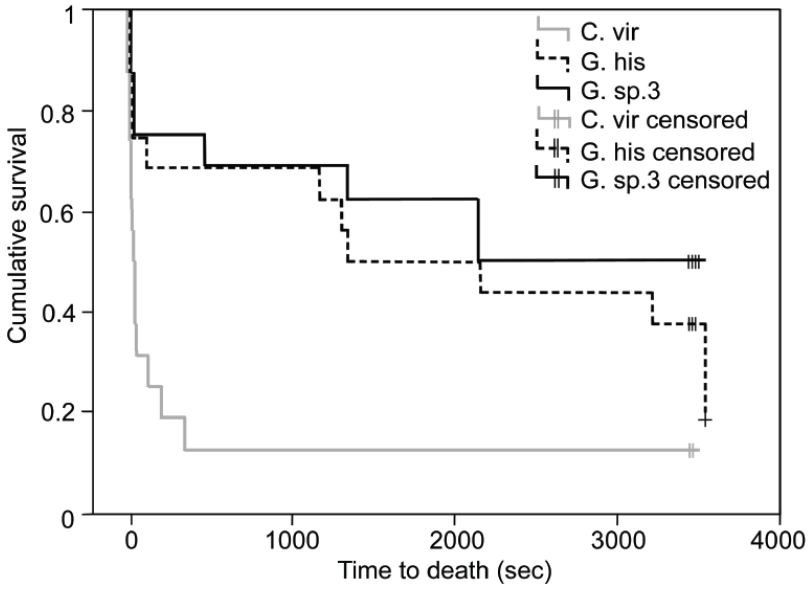

Fig. 12: Kaplan-Mayer estimator in seconds, censored prey species are survivors. Black: *G. histrio*, dark grey: *G.* sp.3, light grey: *C. viridis*. Cumulative survival: y = 1/16 (n = 16 for all predators which consumed prey). Censored specimens indicate survivors.

The longer a coral goby is manipulated in relation to its condition factor, the higher is the probability of getting eaten finally, although the covariate of cumulative manipulation time was not significant. The analysis revealed that the condition factor of prey was strongly positively correlated with the manipulation time of gobies by a predator. Therefore I used the residue of manipulation time in the analysis.

Tab. 7: Cox-Regression for two species of *Gobiodon* (influence of covariates on survival).

covariates	**B**	**df**	**significance**	**Exp(B)**
regurgitation (per hour)	1.67	1	0.001	5.33
condition factor (prey)	1.07	1	0.003	2.91
RES (cumulative MT)	0.003	1	0.074	1.00

Regurgitation (per hour): regurgitations extrapolated on the time-span per hour until eventual death occurred (log10 of sec); condition factor of prey; RES: residue of cumulative manipulation time. B = coefficient B, Exp(B) = exponentiation of the B coefficient (relative risk to die).

6 DISCUSSION

Both species of *Gobiodon* in the Red Sea, *G. histrio* and *G.* sp.3, release skin secretions under circumstances of stress, which efficiently affect the locomotor abilities and even survival of fishes exposed to these secretions. Both potential space competitors and predators are negatively affected when exposed, suggesting that the secretions are toxic. In the course of bioassay studies conducted for studies on species-specific toxicity levels, a possible space competitor, *C. viridis*, exhibited loss of equilibrium when exposed to toxins of both cryptically (*G.* sp.3) and non-cryptically coloured (*G. histrio*) gobies. In contrast, a non-stressed goby shows no negative effect on *C. viridis*, suggesting that only mechanical friction stimulates toxicity release as also suggested by Lassig (1981). Toxicity may also be activated only during secretion, as shown for trunkfishes (Thomson 1964).

Because *G. unicolor*, the cryptically coloured representative used in bioassay studies of Schubert et al. (2003), is closely related to or even identical with *G.* sp.3 based on molecular data (Herler, unpublished data), my study can be well compared with Schubert et al. (2003), who found interspecific differences in toxicity related to colouration, standing in contrast to Hashimoto et al. (1974), who found no remarkable differences between species of *Gobiodon*. In the present study, the cryptically coloured species was also found to be less toxic, although toxicity was not much lower than in *G. histrio*, somehow contrasting the results of Schubert et al. (2003). These differences may be due to distinct geographic regions or varying sensibility of both bioassay species. For the first time it was investigated here how potent toxins of *G. histrio* and *G.* sp.3 are after a certain time. Remarkable differences in toxicity between both *Gobiodon* species were only apparent until 10 minutes (significant differences even only until 1 minute after release) after rubbing while loss of equilibrium times became equal in later experiments. This suggests that predation pressure may be generally high for both coral gobies causing relatively high levels of toxicity for both. However, *G.* sp.3 may be less exposed to predation due to its structurally more complex host coral (Herler, personal communication), leading to less investment into skin toxins since the advantage of toxins must outweigh costly production (Williams et al. 2003, McCormick and Larson 2008). In the present study, all bioassay specimens showed loss of equilibrium when exposed to toxins that were taken from gobies 30 minutes or less before trials started, indicating that toxins are potent within this time-span. After 60 minutes from yielding toxins, the first bioassay species lacked a loss of equilibrium, indicating non-toxicity. This might be due to enzymatic degradation (Hashimoto et al. 1974) or

indicates bacterial detoxification (Thomson 1964). Apart from toxins, oxygen decline may be a factor possibly initiating loss of equilibrium. However, this effect can be excluded since time to loss of equilibrium is increasing with experimental time and does frequently even not occur in the longer intervals. Furthermore, preliminary investigations have shown that keeping *C. viridis* in non-aerated water over 24 hours did not cause mortality.

Toxicity is most common among sessile, slow-moving and site-attached reef inhabitants (Cameron 1974, Bakus 1981), which is possibly linked to high predation pressure. On that account, the main focus of this study was paid on how a *Gobiodon* predator would deal with a coral goby under natural circumstances, rather than carrying out a detailed examination of predator or prey behaviour. Thereby it was shown that post-capture behaviour of predators stands in sharp contrast to pre-capture behaviour, depending on prey species. It was observed that skin secretions of living cryptically and non-cryptically coloured coral gobies function as predation deterrence. As shown in pre-capture events, predators (*E. fasciatus*) were equally interested in all three species, suggesting that gobies may be potential prey species in their natural environment. Approaches were basically performed to draw nearer, since approaches usually start from further distances than strikes. They also served to check prey for suitability, since many approaches were performed without subsequent action, possibly because predators may be able to detect toxins prior to physical contact (Bakus 1981, Schubert et al. 2003). Nevertheless many predators attacked both *C. viridis* and *Gobiodon*. Most captures were performed towards the nearest prey, suggesting that the primary trigger for prey selectivity was prey accessibility (passive selection) rather than the prey species (active selection) (Clements and Livingston 1984). To draw nearer without causing anti-predator responses and to overcome longer distances, predators frequently broke approaches down to multi-stage approaches. The longest distances covered by a predator during approaches or strikes were towards *C. viridis*, because its higher swimming activity (referred to as “moving”) is certainly attracting predators. In contrast, both *Gobiodon* species moved rarely and many even did not exhibit anti-predator-responses if approached or attacked. This behaviour may be caused by their acclimatisation to secure shelter in nature, which was missing during experiments, and by their typically less active life-style, which is both connected to the close association with their coral host. It indicates that coral gobies have had no influence on their predation risk by enhancing anti-predator behaviour (McCormick and Holmes 2006). Most toxicological predator-prey experiments used feeding bioassays with tissue or secretions of toxic organisms rather than living specimens to establish that predation deterrence was not caused by behavioural defence (McQuaid et al. 1999). During

this study, coral gobies did not exhibit relevant anti-predator behaviour and in general, the difference between pre- and post-capture behaviour of the predator was much more important than the predators' reaction on prey behaviour. Predators were also attracted by groups of two or three prey specimens, demonstrated by higher approach rates, because usually groups cause attention more quickly (Botham et al. 2005). Nevertheless, most strike captures were performed towards single specimens, implying that approaches not only helped to come closer but also served for separation of groups, since single specimens are easier to catch.

Despite pre-capture events did not show clear predator preferences, choices for consumption became explicit during post-capture behaviour. *Chromis viridis* was not only consumed most often and clearly was a predator's first choice of prey, but was in almost all cases also consumed immediately without expel after capture. As shown for other toxic or unpalatable species (Bakus 1981, Wiklund and Järvi 1982), both *Gobiodon* species experienced repeated expels after predators sampled preys. Although some died after exceptionally long manipulation times, specimens usually survived regurgitations as confirmed for other species (Bakus 1981, Wiklund and Järvi 1982), providing evidence for a dramatic increase of individual fitness. The level of toxicity is assessed by individual predators relative to their own resistance and prey species that possess the most potent toxins are rejected (Williams et al. 2003). Despite preceding rejections most coral gobies were finally consumed after a time of cumulative manipulation in a predator's mouth, which was accompanied by a high regurgitation frequency. The very soon expel (after a few seconds) following the first capture of gobies signalises either unpalatability or quickly ascertained toxicity or both. Hashimoto et al. (1974) described a stinging and bitter taste when a coral goby is put on the tongue, suggesting distastefulness by all means. In natural environments a predator usually has only one chance to catch a prey. A prey, which is regurgitated after a few seconds of manipulation time, possesses a very high chance of survival. Close associations with the substrate, in particular with a complex structure and many close refuges, as present in the habitat of coral gobies, may further enhance survival (Feary et al. 2007, Wall and Herler 2009).

One would expect that ingestion of *C. viridis* is more difficult compared to coral gobies, because *C. viridis* exhibits full body squamation and robust fin spines. Nevertheless, the cumulative manipulation time to final ingestion of *C. viridis* was much shorter than in gobies. Manipulations in the mouth of a predator are in accord with Lassig (1981), who also noticed that gobies were rarely swallowed without prior manipulation. The repeated expel of *Gobiodon* species and the according prey preferences of predators suggest that chemical deterrence, in

particular if combined with toxicity, is more efficient than mechanical/physical deterrence mechanisms. Despite final consumption and digestion of gobies there was no negative effect on predators apparent even after observations overnight, agreeing with Schubert et al. (2003) and Lassig (1981). This may be explained by the observed coherence between cumulative manipulation times of gobies (about 120 seconds) and the time required for yielding most of the toxins through mechanical friction (about 90-120 seconds), as performed in bioassay studies. I therefore suggest that predators may repeatedly manipulate coral gobies before ingestion to cause release of most of the toxins.

Even though toxins had no apparent effect on *E. fasciatus* after digestion, a predator exposed to skin secretions of gobies suffered loss of equilibrium. This may indicate that the toxins lose their haemolytic property upon digestion and may primarily target the blood circulation via the gills of predators by disruption of the red cell membrane, resulting in haemolysis (Primor and Zlotkin 1975). Boxin for example, related to pahutoxin, achieves its ichthyotoxicity only upon delivery through the water column, suggesting that it affects externally allocated targets (Kalmanzon and Zlotkin 2000). However, further research is needed to address the true nature and physiological function of *Gobiodon* toxins.

Predator preferences may not only be influenced by distance or olfactory stimuli but colouration differences between preys may as well influence a predator's choice. However, it was previously shown that feeding preferences are usually not influenced by colouration (Bakus 1981, Dunlap and Pawlik 1996). Further, predators appeared to prefer prey with high condition factors, as revealed by the Cox-regression. Because of the negative correlation between condition factor and total body length, I suggest that small gobies are at a higher risk of being eaten. This might be due to easier handling of smaller fish, but possibly also because of lower toxicity levels since there was also a significant negative correlation between length and toxicity in *G. histrio*. Another factor possibly affecting a predator's choice of prey during experiments may be its potential naivety towards the prey, since results may be biased if a predator had previous encounter-experiences with one or more of the experimentally used prey species. Nevertheless, it has been shown in this study that, although predators showed a preference for prey other than gobies, they do consume gobies even in the first place and continue consuming a second and sometimes a third prey, suggesting that predators are still stimulated by gobies and are keen on consuming them. Thereby, the post-capture treatment of preys by predators during experiments may reflect very well how predators would possibly deal with a coral goby in nature. If gobies are subject to predatory attacks without protection, as it was shown in the

course of this study, they may be eaten even though they possess such strong defensive chemical deterrence. Thus, a noteworthy proportion was finally consumed after repeated regurgitations and long manipulation time. They appear to release a harmful dose of toxins after intensive manipulation, and individuals of *G. histrio* required about 48 hours to rebuild toxic skin secretions, although not as highly potent as in the primarily release of toxins, as I have shown in the course of this study. Therefore the coral host may be an important refuge. I suggest that their close attachment to a coral host may be essential by providing additional protection.

One of the possible causes of the evolution of toxicity of coral gobies may be migration, although it occurs rarely in all life-stages (Feary et al. 2007, Wall and Herler 2009). During early life stages where they lack the secure shelter of a certain host coral colony, a phase generally characterised by high mortality rates as naïve individuals enter new habitats (Carr and Hixon 1995, Alamany and Webster 2006), coral gobies frequently move between corals (Wall and Herler 2009). They may also move if they outgrew the coral colony size, as shown for other coral-dwelling reef fishes (Belmaker et al. 2009). The relation between toxicity and migration leads to the conclusion that *G.* sp.3 may be less toxic than *G. histrio* due to its lower migration rate between corals (Herler, personal comment), thereby leading to less investment into skin toxins. Arising questions, such as if gobies in areas of isolated reef patches are more toxic than in continuous reefs, where migration may be less dangerous, or if toxins play a possible role in deterring larval settlement, a process that would be important for the defence against space competitors, are needed to be solved.

In general, more toxicological research (chemical and biomolecular) on toxins of *Gobiodon* needs to be performed to determine the nature of the toxins, since two different toxin classifications have been proposed by Hashimoto et al. (1974): grammistin, a simple peptide (Sugiyama et al. 2005), or pahutoxin, a choline ester with fatty acids (Shiomi et al. 2000) and being not effective by intramuscularly injections (Kalmanzon and Zlotkin 2000). The biologic activity of the toxin (effect on animals of different phyla, such as Crustaceans and other coral inhabitants) should be tested, as done for boxfishes (Thomson 1964), as well as lethal concentration (LC_{50}) values have to be investigated. Finally, more field research and experiments are necessary to evaluate additional roles the toxins may hold within the environment of coral gobies of the genus *Gobiodon*, as assumed above.

7 ZUSAMMENFASSUNG

Ein grundlegendes Ziel dieser Arbeit war es herauszufinden, ob lebende Korallengrundeln der Gattung *Gobiodon* als Beute in Frage kommen, insbesondere dann, wenn alternatives Nahrungsangebot besteht, und ob sich hinsichtlich zweier unterschiedlicher *Gobiodon*-Arten (eine auffällig und eine kryptisch gefärbte Art) eine Präferenz des Prädators abzeichnet. Im zentralen Interesse standen dabei die Hauttoxine der Korallengrundeln. Bioassay-Untersuchungen mit *C. viridis* als Bioassay-Art, welche zum einen Aufschluss über die Giftstärke und zum anderen über interspezifische Unterschiede zeigten, haben ergeben, dass die kryptische Art, *G.* sp.3, weniger starke Hauttoxine besitzt, obwohl der Toxizitätsunterschied zu *G. histrio* nicht groß und vor allem nur von kurzer Dauer war. Zehn Minuten nach der Giftabgabe war der Unterschied vernachlässigbar und durch die stetige Wirkungsabnahme bei beiden Arten zeigte sich nach 60 Minuten die erste Wirkungslosigkeit des Toxins, welche nach 120 bzw. 240 Minuten häufiger wurde. Damit wurde zum ersten Mal getestet, wie lange das Gift von *G. histrio* und *G.* sp.3 wirksam bleibt. Die potentielle Giftwirkung, die Giftstärke und die Wirkungsdauer des Giftes unterstützen die Vermutung, dass der Prädationsdruck außerhalb der Wirtskoralle stark ist, wobei *G.* sp.3 einen höheren Schutz durch seine strukturell komplexere Koralle genießt, was mit der geringeren Basistoxizität in Zusammenhang stehen könnte.

Es hat sich gezeigt, dass die meisten Räuber ein grundsächliches Interesse an allen drei angebotenen Beutefischen hatten (*C. viridis*, *G. histrio*, *G.* sp.3), verdeutlicht durch das Beute-Annäherungs-Verhalten der Räuber. Annäherungen an die Beute (engl.: approaches), sowie Attacken (engl.: strikes) erfolgten artunspezifisch. Mit Ausnahme von *C. viridis*, welcher häufig aus großen Distanzen angenähert wurde, waren die Nähe der Beute zum Prädator (am häufigsten wurden nahestehende Beuteindividuen angegriffen) und auf Gruppierungen der Beute (Annäherungen erfolgten zumeist bei Gruppen) ausschlaggebender für eine Annäherung bzw. einen Angriff. Eindeutige Unterschiede bezüglich der Präferenz haben sich erst beim Fressen bzw. nach der Aufnahme ins Maul verdeutlicht, denn die ungiftige Kontrolle wurde trotz starkem mechanischen Schutzstrukturen (beschuppter Körper, harte Flossenstrahlen) mit wenigen Ausnahmen gleich bei ihrer ersten Aufnahme gefressen. Von insgesamt 54 angebotenen Fischen wurden 33 (14 *C. viridis*, 11 *G. histrio* und 8 *G.* sp.3) von insgesamt 16 Prädatoren gefressen. Von den 16 Beutefischen, welche als erstes gefressen wurden, waren 10 (63%) *C. viridis*, 4 (25 %) waren *G. histrio* und 2 (13%) waren *G.* sp.3. Während nur zwei *C. viridis* ausgespuckt wurden, wurde die große Mehrheit der beiden Korallengrundelarten nach

mehrmaliger kurzer Manipulationszeit im Maul des Räubers wiederholt ausgespuckt. Die Manipulationszeit pro Aufnahme im Maul des Räubers blieb dabei bei allen 3 Beutefischen gleich war. Im Unterschied dazu war die Manipulationszeit der ersten Aufnahme im Maul stark variabel zwischen dem Kontrollfisch, mit langer Dauer (Median (unteres, oberes Quartil) in Sekunden: 25 (7, 32)), und den beiden Korallengrundeln, bei denen sie nur wenige Sekunden dauert (*G. histrio*: 7 Sek. (4, 28), *G.* sp.3: 4 Sek. (1, 45)). Die Korallengrundeln weisen im Gegensatz zu *C. viridis* (26 Sek. (7, 68)) auch eine höhere kumulative Manipulationszeit bis zum Tod bzw. Gefressen-werden auf (*G. histrio*: 89 Sek. (45, 212), *G.* sp.3: 107 Sek. (9, 174)), welche sich aus dem wiederholten Ausspucken ergibt. Ab einem bestimmten Wert (100 Sekunden bei *G. histrio*, 54 bei *G.* sp.3) der kumulativen Manipulationszeit erhöht sich diese auffallend bei ca. der Hälfte der Individuen beider Meergrundelarten.

Im natürlichen Umfeld würde das bedeuten, dass eine Korallengrundel nach dem ersten Fressversuch meist entkommen könnte und damit bedeutend höhere Überlebenschancen im Falle eines Angriffes durch einen Räuber aufweist. Dass etwa die Hälfte aller Korallengrundeln letztendlich dennoch gefressen wurde, liefert eine mögliche Erklärung dafür, warum *Gobiodon* Arten trotz ihrer Giftigkeit den Schutz ihrer Wirtskoralle genießen. Denn prinzipiell scheinen Korallengrundeln eine durchaus interessante Beute für einen Räuber zu sein und insbesondere durch Hunger motivierte Räuber betreiben sogar einen hohen Aufwand durch mehrmaliges Aufnehmen und Manipulieren im Maul, um die Fische fressen zu können. Unklar bleibt aus welchem Grund die Fische gleich nach dem ersten Fressversuch wieder ausgespuckt und erst so lange manipuliert werden müssen, bevor sie konsumiert werden. Es wäre möglich, dass die Grundeln neben ihrer Toxizität auch schlecht schmecken - ein Umstand, auf den schon Hashimoto et al. (1974) aufmerksam machte – und der Geschmack und nicht die Toxizität der primäre Auslöser für das Zurückweisen ist. Dabei wäre es naheliegend, dass das Hauttoxin über den Verdauungsweg seine Giftigkeit verliert, wie im Fall von Schlangengift (Pawlowsky 1927). Des Weiteren wäre es anhand der dargelegten Ergebnisse denkbar, dass eine Grundel zumindest den Großteil ihrer Giftigkeit durch die mechanische Manipulation im Mund eines Prädators verliert. Ein Hinweis darauf ist, dass ein Zusammenhang zwischen jener Dauer, bis zu der eine Korallengrundel durch manuelle Manipulation ungiftig wird (90 bis 120 Sekunden), und der Manipulationszeit, welche eine Korallengrundel im Mund eines Prädators verbringt bevor sie gefressen wird (ca. 60 bis 120 Sekunden), besteht.

Eindeutig geht aus dieser Arbeit sich jedenfalls hervor, dass toxische Abwehrmechanismen bzw. schlechter Geschmack einen Vorteil gegenüber physikalischen

Schutzstrukturen (z.B. Beschuppung und harte Flossenstrahlen) bieten, denn selbst gegebenenfalls, dass *Gobiodon*-Individuen den Schutz ihrer Wirtskoralle verlassen, überleben sie, im Gegensatz zu Riffbarschen (*Chromis*) ,eine oder mehrere räuberische Attacken in den meisten Fällen.

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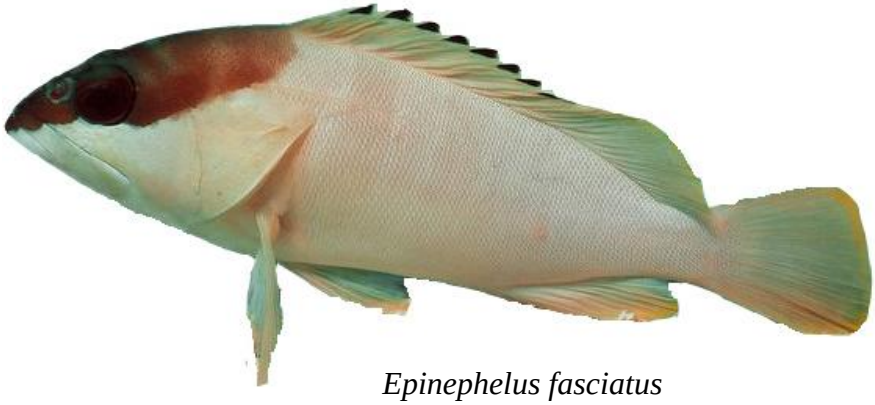
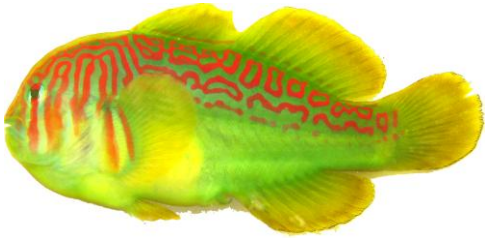
9 APPENDIX

Species used for all experiments:



Epinephelus fasciatus

Chromis viridis



Gobiodon histrio

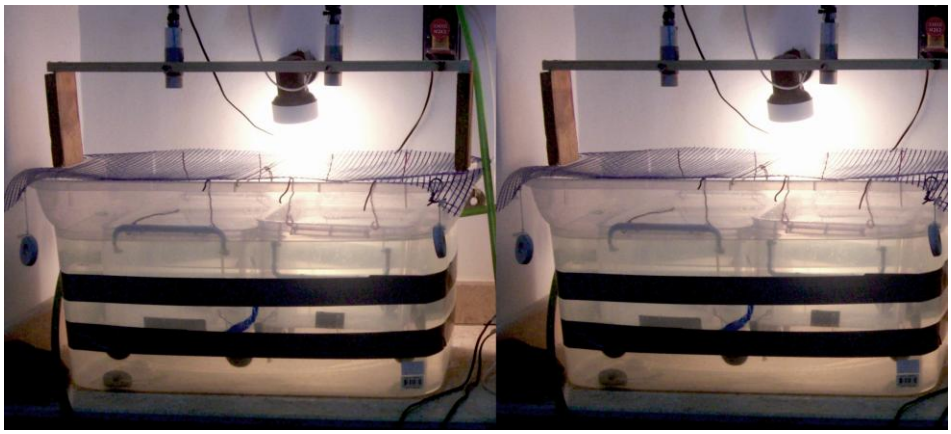
Gobiodon sp.3

Bioassay – experiment:

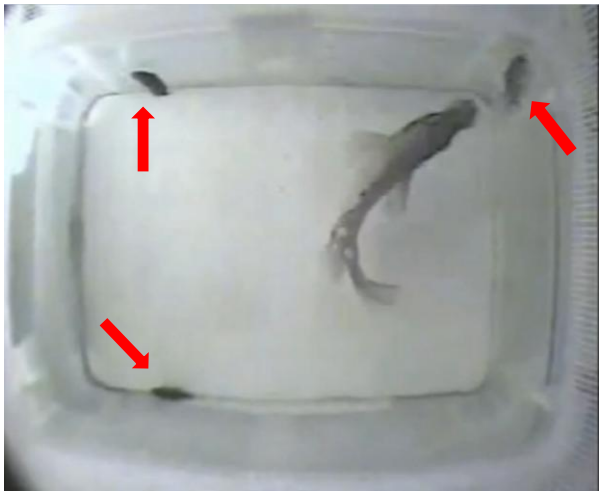
Rubbing of *G. histrio* (red arrow) in 10ml seawater inside a plastic bag. Note the milky, foamy water.

Measurements of time to loss of equilibrium of *C. viridis* inside buckets, which contain skin secretions of *Gobiodon*.

Predator preference – experiment:



Aquarium setup for video analysis, showing two tanks with two containers inside each and cameras inside base frame above.



Example picture for analysing video recordings. In the middle: *E. fasciatus*, right: *C. viridis*, top left *G.* sp.3, bottom left: *G.*

Meer

Wenn man ans Meer kommt
soll man zu schweigen beginnen
bei den letzten Grashalmen
soll man den Faden verlieren

und den Salzschaum
und das scharfe Zischen des Windes einatmen
und ausatmen
und wieder einatmen

Wenn man den Sand sägen hört
und das Schlurfen der kleinen Steine
in langen Wellen
soll man aufhören zu sollen
und nichts mehr wollen wollen, nur Meer
Nur Meer

Erich Fried

10 Danksagung

Rückblickend betrachtet habe ich durch die Diplomarbeit sowohl auf naturwissenschaftlicher, als auch auf persönlicher Ebene sehr viel dazu gelernt. Da sich eine Abschlussarbeit jedoch kaum alleine bewältigen lässt, möchte ich mich an dieser Stelle bei meinen Unterstützern und Helfern bedanken.

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Der nachhaltigste Dank gilt Dominik für Resonanz & die Ermutigung jeden einzelnen Tag.

Schön, dass es dich gibt!

So many foreign worlds (So relatively fucked) *So ready for us*

11 CURRICULUM VITAE

Personal information

Name	Barbara Gratzer
email	barbaragratzer@gmail.com
Nationality	Austria
Date of birth	6-2-1985

Education

2010-2011	Diploma thesis at the institute of Integrative Zoology, University of Vienna (marine fish ecology)
2004 up to now	Studies of ecology with main focus on marine biology at the University of Vienna, Austria
09/1999 – 06/2003	Katholisches Oberstufen Realgymnasium Kettenbrücke
09/1994 – 07/1999	High School in Steinach am Brenner

Research activities

2010	Field research for diploma thesis in coral reefs of Red Sea (underwater fieldwork with SCUBA included)
2010	Stabile isotope course
2009/10	Marine practical work in the zoo "Haus des Meeres" (nursery of animals and scleractinian corals, water quality measurements)
2009	Marine turtle field course in Yaniklar, Turkey
2008	Marine practical course in the Red Sea: artificial reefs (underwater fieldwork with SCUBA included)
2007	Marine biological field course on the Mediterranean fauna and flora in Rovinj, Croatia
2007	Marine ecology practical course in STARESO, Calvi, Corsica (underwater fieldwork with SCUBA included)
2005	*Acanthaster planci* - campaign, Tioman Island, Malysia

Work experience

2005 – 2010	Waitress
2003	Secretary for "Bofrost", Switzerland
2002	Temporary personnel and laboratory work for "Syngenta", Switzerland

Personal skills

Languages	English (very good), French (little)
Licences	diving: PADI RESCUE (135 dives) driving: car and motorcycle
Software	MS Word, MS Excel, MS PP, Adobe Ps, Adobe Ai, Past
additional	organisation of high school prom (3000 guests) president of the school union stay abroad in Australia, New Zealand, Thailand, Malaysia and Seychelles