Final Draft
of the original manuscript:

Helmholz, H.; Johnston, B.D.; Ruhnau, C.; Prange, A.:
Gill cell toxicity of northern boreal scyphomedusae Cyanea capillata and Aurelia aurita measured by an in vitro cell assay
In: Hydrobiologia (2010) Springer

DOI: 10.1007/s10750-010-0216-9

Gill toxicity of northern scyphozoan species *Cyanea capillata* and *Aurelia aurita* measured by an in vitro cell assay

Heike Helmholz1, Blair D. Johnston2, Christiane Ruhnau1, Andreas Prange1

1 GKSS Research Centre, Inst. f. Coastal Research, Dept. f. Marine Bioanalytical Chemistry, D-21502 Geesthacht, Germany

2 Ecotoxicology and Aquatic Biology Research Group, School of Biosciences, University of Exeter, Hatherly Laboratories, Prince of Wales Road, Exeter, UK EX4 4PS

Corresponding author
Heike Helmholz
GKSS Research Centre
Institute for Coastal Research
Department: Marine Bioanalytical Chemistry
Max-Planck-Street 1
D-21502 Geesthacht
Germany
Email: heike.helmholz@gkss.de
Tel: 0049 – 4152/871844
Fax: 0049 – 4152/871875

Key words: jellyfish, RTgill-W1, venom, toxin

This paper has not been submitted elsewhere in identical or similar form, nor will be during the first three month after its submission to Hydrobiologia.

Abstract

Scyphozoa as a major component of gelatinous zooplankton communities express successful foraging behaviour and may impact fish larvae and small fish especially when they occur in masses. The predation impact and severe toxic effects on fish are still under investigation. Therefore, a rainbow trout gill cell line is utilized as an indicator for ichthyotoxic effects. A sensitive *in vitro* toxicity assay was developed and applied for the quantitative determination of the potential gill toxicity of dominant jellyfish species of the North Sea.

The present investigation compares the toxic potential of the Lion's Mane jellyfish *Cyanea capillata* and the Moon Jelly *Aurelia aurita*, the differentiated toxigenic organs and various organism size classes. Cultures of gill cells were exposed to whole venoms at increasing protein concentrations. Dose-dependent morphological changes in cell growth as well as viability responses of the affected cells are documented. Morphological changes can be observed within 1h after treatment. Detachment, clumping and lysis of cells occur in a dose dependent manner. The damaging effect on gill cells is quantified as relative toxicity by measuring the enzymatic conversion of a fluorescent dye. In general, a decrease in the metabolic activity of the cells can be detected above a protein concentration of 2.0 µg mL^{-1} and a total loss of activity is observed above 40.0 µg mL^{-1}. Gel electrophoresis showed the high complexity of venoms and the broad molecular weight range of the containing proteins.

It was shown that the developed *in vitro* assay for cytotoxic effects against gill cells is a useful tool for investigating the potential ichthyotoxicity of jellyfish.

Introduction

The investigated scyphozoan species *Aurelia aurita* and *Cyanea capillata* belong to the most abundant jellyfish species in the North Sea region (Gröndahl, 1988; Bamstedt et al., 1994). As a member of the phylum cnidaria their main characteristic is the proliferation of specialized cells (cnidocytes) harbouring cnidocysts as cell organelles which contain a complex mixture of highly active and structural diverse toxins. The cnidocyst capsules are discharged as a response to adequate chemical and mechanical stimuli as excited by prey organisms. The toxin mixture released as whole venom induces different effects on prey e.g. paralysis. The development and distribution of certain types of cnidocytes and the injection of bioactive compounds is a crucial factor for prey capture and digestion by cnidarians (Regula et al., 2009; Kintner et al., 2005).

The ecological impact as gelatinous predators on zooplankton species and fish fry is getting more attention since an increase in jellyfish biomass and abundance has been predicted (Hay, 2006). Causes and consequences of such an increase are under discussion. Factors such as over-fishing, new polyp settlement areas and climate changes could play an important role (Purcell et al., 2007). Negative effects of a higher abundance of gelatinous zooplankton on fish recruitment are described in some publicatons (Bamstedt, 1990; Bamstedt et al., 1994; Malej et al., 2007; Barz & Hirche, 2007; Omori et al., 1995; Moeller & Riisgaard, 2007; Titelman et al., 2007; Behrends & Schneider, 1995; Lynam et al., 2005; Lynam et al., 2006).

In order to assess the ecological role of jellyfish in the food web a number of *in vivo* experiments with different types of potential prey organisms has been performed (Bamstedt & Martinussen, 2000; Martinussen & Bamstedt, 2001; Bailey & Batty, 1984; Hansson, 2006). The direct outcome of feeding rates, feeding behaviour and prey selection is advantageous for modelling the role of jellyfish in the food web (Suchman & Sullivan, 2000; Sullivan et al., 1997). In contrast, disadvantages of such i*n vivo* experiments are the necessity of hatching facilities and the maintenance of living organisms.

Besides their ecological impact, the stinging capacity of scyphozoa is a field of ongoing concern in particular in tropical and subtropical regions were lethal species can be found. Envenomations in northern coastal areas are considered to be much less serious, but with a northward shift of Mediterranean species and a predicted higher occurrence an increase of severe stings can be expected (Mills, 2001).

Whereas *A. aurita* is harmless for humans, *C. capillata* can cause severe dermal irritations and pain by accidental contact. There are only a few studies concerning the toxic potential of *Aurelia aurita* and no detailed biochemical information about the composition of the venom is available (Burnett et al., 1988; Radwan et al., 2001; Segura-Puertas et al., 2002). The stinging capacity of *Cyanea capillata* is described as strong and a few in vitro and in vivo studies are available investigating the toxic potential of *C. capillata* venoms (Rice & Powell, 1972; Walker, 1977a; Walker, 1977b; Walker et al., 1977; Fenner & Fitzpatrick, 1986; Long & Burnett, 1989; Helmholz et al., 2007). A strong hemolytic and cytotoxic as well as a cardiotoxic activity could be demonstrated. The analysis of the proteinaceous components of the venom showed a spectrum of proteins in a broad molecular size range, but no structure-effect relationships have been determined.

Since the cell damaging detected in vitro assays can be serve as an indicator for the potential toxic effects and consequently for prey consumption, cell-based approaches are useful tools for a comparative and sensitive toxicological venom analysis. Often the hemolysis of erythrocytes of different animal species or damaging effects on cell lines e.g. hepatocytes are used as responsive elements. Due to the fact that gills are very sensitive to the action of cnidarian venoms, an *in vitro* assay utilizing a gill cell line as target has been established. It has been shown in the literature that this cell line is a valuable tool in ecotoxicological studies investigating the effect of anthropogenic hazardous compounds like polycyclic aromatic hydrocarbons (PAHs), metals and also of waste water (Schirmer et al., 1998; Lee, et al., 2008; Dayeh et al., 2002; Dayeh et al., 2005). But to our knowledge this rainbow trout gill cell line has not been used for the detection and assessment of toxic effects of natural marine substances. In this publication, the sensitivity of gill cells against crude scyphozoan venoms is demonstrated. The developed *in vitro* assay is introduced as a complemental approach to *in vivo* studies. Besides the quantitative determination of gill toxicity, it generally offers also the possibility to clarify cellular responses and modes of action on gills of jellyfish toxins.

Materials and methods

Material

Leibovitz L15 Medium (L15) (Lonza group, Wuppertal, Germany), penicillin/streptomycin solution and phosphate buffered saline (PBS) (10x) were purchased from Invitrogen (Karlsruhe, Germany). L-glutamin, Bradford reagent, bovine serum albumin (BSA)

fraction V were obtained from Sigma (Munich, Germany) and foetal calf serum (FCS) from PAA Laboratories (Cölbe, Germany).

Organisms

Jellyfish of the species *Cyanea capillata* (L.) and *Aurelia aurita* (L.) were collected at research cruises to the Scottish Orkney Islands and Western Isles in June 2006 and 2008 and at the German Baltic Sea Coast in October 2006 and 2008. The animals were separated into groups (Tab.: 1 and 2) according to the average umbrella diameter. Mesenteric and fishing tentacles were sliced immediately after sample collection and used for subsequent processing.

Preparation of cnidocysts

Fishing and mesenteric tentacles were used for the preparation of intact cnidocysts. The preparation was performed in thermostated laboratories within 24 h. The material was stirred gently in distilled water on ice. *A. aurita* tissue material was not stirred. The relation of organic tissue and distilled water was approximately 1:5. After 10 h the suspension was filtered through a nylon sieve (500 µm mesh size) to discard the mesoglea residue. The filtrate was centrifuged at 4°C for 5 min at 3000 rpm. After discarding the supernatant the residues were washed 2-3 times with sterile-filtered seawater. The content, purity and integrity of cnidocysts were controlled microscopically and the cnidocysts concentrate was stored at -80°C until further use.

Cnidocyst lysis and protein extraction

Before lysis the isolated cnidocysts were suspended in ice cold 10mM acetate buffer pH 5.5 and discharged in a cooled sonicator (Branson Sonifier 450, G. Heinemann Ultraschall- und Labortechnik, Schwäbisch Gmünd, Germany). The suspension was centrifuged at 11000 rpm for 5 min at 4°C. The supernatant was carefully removed, sterile filtrated and used for protein determination. The protein content was measured by Bradford reagent in a protein microassay with BSA as standard protein (Bradford, 1976).

These protein extracts were immediately used for bioactivity assays or stored at -80°C until further use. The ratio of discharged capsules in the remaining residue was counted microscopically.

Cell toxicity assay

A cell viability assay (CellTiter-Blue®, Promega, Mannheim, Germany) was used to detect the acute toxicity of the extracts. A rainbow trout cell line RTgill-W1 ATCC No: CRL-2523 (LCG Promochem, Wesel, Germany) was cultivated in Leibovitz L15 cell culture medium with 10 % FCS, 2 mM L-Glutamine, 100 IU penicillin and 100 µg streptomycin per mL medium at 20°C. Cells of a continuous culture were sowed into a black microtiter plate in a density of 10^4 cells per well in 75 µL L15 medium without FCS and allowed to settle and reattach for 24 h. After this adaptation step 25 µL of extracts were applied to each well in protein concentrations of 0.1 - 4.0 µg per well (corresponding to 1 – 40.0 µg mL^{-1}). Extracts were diluted with L15 medium. The following controls were used for the detection of the relative toxicity of the extracts: a cell positive control growing in 100µL L15 medium, a negative control without cells and a buffer control containing the corresponding volume of 10 mM acetate buffer. After an incubation of 48 h at 20°C, 20 µL of CellTiter-Blue® reagent was added and the fluorescence intensity of the metabolized resorufin was recorded after 4 h incubation at 20°C at 560excitation/590emission (Victor 3, 1420 Multilabel Counter, Perkin Elmer, Rodgau - Jügesheim, Germany).

The assay was performed with eight replicates of each extract concentration and controls and the experiments were repeated independently at least three times. The percentage of vital cells was calculated by setting the absorbance of positive controls minus the values of no-cell control 100%. Mean and standard deviation values were used for statistical analysis (software: WinStat). The U-Test (Mann-Whitney test for comparison of 2 data sets) was applied and significance is indicated as $P < 0.05$.

Gelelectrophoresis

Tricine-sodium dodecyl sulfate-gelelectrophoresis was performed with self-casted polyacrylamide gels according to (Schaegger & von Jagow 1987). A 10 % separating gel was combined with a 4 % stacking gel. Dimensions of the gel were 20 x 20 cm. A SERVA unstained SDS Page protein marker 6.5-200 kDa (Serva electrophoresis GmbH, Heidelberg, Germany) was used for the estimation of molecular weights. The one dimensional electrophoresis was performed with the Protein II electrophoresis system (BioRad, Munich, Germany). After silver staining (Blum et al., 1987), the gels were analyzed with the Bio Imaging System Genegenius (Syngene, Cambridge, UK). Molecular weight determination is based on at least four independent SDS Page experiments.

Results

Development of the gill cell toxicity assay

Cells of the continuous culture of RTgill-W1 were grown in Leibovitz L15 medium with 10% FCS at 20°C according to the recommendations of Bols et al., (1994) and under our laboratory conditions they reproducibly developed a dense monolayer of cells with a polygonal, epithelial-like shape (figure 1).

After exposure to whole jellyfish venom at different protein concentrations, cells respond with changes in morphology and growth. Morphological changes can be observed 1 h after treatment and become more obvious within the 48 h of incubation. Detachment, clumping and lysis occur in a dose-dependent manner (figure 2).

Even though the cell damage is already obvious during microscopic examination, a quantification of cell viability has to be performed in order to compare and assess the gill toxicity. The utilized CellTiterBlue® assay is a homogenous, fluorometric method for estimating the number of viable cells by measuring their metabolic capacity. Non-viable cells lose the capacity to reduce the indicator dye (resazurin). The enzymatic transformation of the fluorescence dye and thus the intensity of the fluorescence signal depend on cell vitality, cell proliferation and on the dye incubation time.

Since the commercial cell viability test was developed and applied for mammalian cells the test conditions have to be adapted to the chosen culture conditions which are optimal for fish cells. Due to the relative slow growth of the gill cells, the recommended cell number has been reduced to 10^4 cells per well in order to increase the sample throughput. This initial cell number is on one hand sufficient to form interacting cell aggregates during the exposure period and the growth is not limited by nutrients and space. On the other hand, it is high enough for a fluorescence signal detection showing clear differences between controls and disturbed cells.

In order to ensure a reliable performance of the assay, the fluorescence signal of the positive cell control is used as an indicator for RTgill cell vitality over a continuous cultivation period with regular subculture procedures. After a phase of three months with a relatively homogenous metabolic activity, a slight but consistent decrease of the fluorescence signal can be observed and a resuscitation of a cryo-preserved cell charge is recommended.

Within this stable period a variation coefficient of 8.5 % (n = 341) is achieved. Therefore, a loss of cell viability of 10 % due to the impact of jellyfish venom is judged as a true toxic effect.

Gill toxicity of *Aurelia aurita* venoms

Whole venoms of fishing and mesenteric tentacles obtained from an enriched suspension of all types of cnidocysts induce a concentration-dependent loss of RTgill cell viability. Extracts of mesenteric tentacles show a stronger damaging potential with an effective concentration range of 1.0 µg mL^{-1} up to 20.0 µg mL^{-1} compared to extracts of fishing tentacles (2.0 µg mL^{-1} – 40.0 µg mL^{-1}). Figure 3 shows the comparison of the dose dependent toxic effect of *Aurelia aurita* venom (Sample A. aur 2) from mesenteric and fishing tentacles exemplarily. The effective concentration range was chosen according to preliminary experiments between a no-effect concentration and a concentration at which an almost total loss of cell viability could be achieved. At least 4 different protein equivalent concentrations have been applied for each venom sample in order to cover the effect range. Samples were taken from the west coast of the Isle of Lewis/Western Isles and at Mainland/Orkney Islands. Although they were collected in different years treatment and sample preparation was identical (Tab.: 1).

The average umbrella diameter of individuals from the Orkney Islands in 2008 was smaller compared to those from Lewis. Also a reduced toxic effect can be seen for both size groups from the Orkney Islands by comparing the gill toxicity of 10 µg mL^{-1} fishing tentacle venom (Fig.: 4) and for 5 µg mL^{-1}mesenteric tentacle venom (data not shown), respectively.

A significant dependency of gill toxicity on umbrella size could be observed at a concentration of 5.0 µg mL^{-1} mesenteric tentacle venom and 10.0 µg mL^{-1} fishing tentacle venom. Whereas the fishing tentacle venoms of the larger animals are more active than from the animals < 10 cm bell diameter (Fig.: 4) the opposite case can be observed for the crude venom of oral arms.

Gill toxicity of C*yanea capillata* venoms

The application of *Cyanea capillata* venoms obtained by preparation of cnidocysts from mesenteric and fishing tentacles induce similar morphological changes in gill cell growth as it can be observed after exposure to *Aurelia aurita* venom.

Toxic effects can be observed microscopically and measured via the cell viability fluorescence assay at dose above 1.0 µg mL^{-1} crude venom of mesenteric tentacles and above 5.0 µg mL^{-1} crude venom of fishing tentacles indicating an increased gill cell toxicity of oral arms compared to fishing tentacles as it has been observed for *Aurelia aurita* venoms.

Regional differences can be documented comparing samples from the coast of Rousay/Orkney Islands (North Sea) and two different sampling sites at the Baltic Sea (Tab.: 2). The viability of RTgill cells is reduced at a fishing tentacle venom concentration of 10.0 µg mL^{-1} to 2.7 % by *C. capillata* with a mean diameter of 21.5 cm sampled at the Orkney Islands which is a significant stronger toxic activity than the one observed for the samples of the same organismal size from *C. capillata* samples collected at the Baltic Sea coast (39.9 % cell viability C. cap 3 and 12.6 % cell viability C. cap 4). The same regional variation in the toxic potential against gill cells can be observed for the venom of mesenteric tentacles at a concentration of 2.0 µg mL^{-1} (Tab.: 2).

Significant size dependency of gill cell toxicity could be observed after exposure to the crude venom of fishing tentacles at a concentration of 2.0 µg mL^{-1} showing an increased activity of the smaller individuals compared to the individuals with a mean umbrella diameter of 33 cm. Whereas no (sample C. cap 2) or only a slight toxic effect of 17 % (sample C. cap 5) could be detected, the smaller individuals induce a reduction of the cell viability up to 30 %.

Since samples of *Aurelia aurita* and *Cyanea capillata* could not be taken from the same region, a comparison of the gill toxic potential of the two species is difficult. But the values for the relative toxicity indicated that the venom of *C. capillata* (> 30 cm) fishing tentacle express a threefold increased toxic activity and a twenty fold increased toxic activity (*C. capillata* > 20 cm) compared to *A. aurita* of equivalent size classes. The venom of the mesenteric tentacles express a five fold elevated toxic activity of *C. capillata* compared to *A. aurita* for both size classes at a concentration of 5.0 µg mL^{-1}.

Comparative analytical gelelectrophoresis

A comparing overview of the protein content in the crude venoms could be obtained by one dimensional gel electrophoresis (fig 5 A and B). Proteins of a broad molecular range from 6 kDa up to 170 kDa could be detected. Venoms extracted from *Aurelia aurita* showed a higher complexity compared to venoms of *Cyanea capillata*.

An increased number of protein bands can be observed in venoms from larger *A. aurita* reflecting a higher degree of venom complexity. Molecular weight ranges that seem to be highly conservative can be found at 6.5, 13, 17 and 23 kDa. The clusters at 30-37 kDa, 41-47 kDa and 51-59 kDa are remarkable since a pattern of 4 protein bands with a difference of 2000 Da can be detected.
Comparing the venoms from mesenteric and fishing tentacles from *Aurelia aurita* the appearance of a cluster of 4 proteins at 95, 100, 116 and 140 kDa is remarkable. This distinct pattern can not be found in the extracts of fishing tentacles of *A. aurita*.
Regional variations can be detected for the venoms of *Cyanea capillata* with a higher complexity in the samples from the Baltic Sea. Venoms extracted from fishing tentacles showed an increased number of protein bands compared to extracts of oral arms; for example proteins in a molecular weight range of approximately 70 kDa can not be found in venom samples of mesenteric tentacles.

Discussion

Due to the expected future increase in the occurrence of gelatinous zooplankton, their ecological effects on prey organism and the multitude of their possible modes of action, the investigation of the toxic potential and causative substances of dominant scyphozoan species becomes more and more important. Besides the classical laboratory and field experiments for the analysis of gut contents, prey selection, feeding behaviour and feeding rates also in vitro cell-based approaches have been utilized in order to clarify modes of action in different prey organisms and to compare the toxic potential of different species. Conventionally, whole venoms are applied and tested in hemolysis and cell assays with mammalian cells. Here, the application of fish gill cells as a major responsive element indicating ichthyotoxicity of jellyfish has been introduced. Although the feasibility of such a fish cell line for ecotoxicological studies of pollutants has been shown, the effects of natural marine toxins on these gill cells have not been tested. Therefore, the detected toxic potential of the northern scyphozoan species *Aurelia aurita* and *Cyanea capillata* on gill cells can only be discussed in relation to other hemolysis and mammalian cell assays.
The species *Aurelia aurita* is ubiquitous distributed in coastal areas and its ecological impact on pelagic prey organisms is especially relevant when they occur in high abundances (Möller, 1980; Lynam et al., 2005). Since there are no severe envenomations, biochemical investigations concerning the composition of the venom and toxicological characterization of stinging capacity are rare. In vivo experiments with proteinaceous

extracts administered to rats showed an induction of paralysis symptoms and glomerulonephritis (Wiersbitzky et al., 1973). A muscle depolarization indicating a sodium channel activity has been observed for a protein extract of whole *Aurelia aurita* specimen (Kihara et al., 1988). But in both studies no further biochemical information on causative toxins can be found. A more unspecific lytic effect is suggested when red-tide flagellate species are treated with *Aurelia aurita* autolysate (Hiromi et al., 1997). More comprehensive studies have been made by (Segura-Puertas et al., 2002). Beside dermonecrotic activity, also hemolysis and neurotoxicity could be observed for whole venoms of tentacular margins. Lytic effects on human erythocytes have been shown at concentrations above 400 $\mu g\ mL^{-1}$. In comparison to the applied concentration in the present gill cell assay of 1 - 40 $\mu g\ mL^{-1}$ the high susceptibility of the gill cells and the better sensitivity of the gill cell bioassay could be documented. Also size exclusion chromatography and gel electrophoresis of the bioactive venom was performed in the study of Segura-Puertas et al., (2002) indicating toxic proteins at 45 and 66 kDa which matches to detected values in the present investigation. The broad molecular weight range as presented in figure 5 reflecting the complexity of whole venoms is also shown by Radwan et al., (2001). Here, major protein bands at 50, 66 and 100kDa have been shown. But peptides in the lower molecular weight range, as shown in this study at figure 5, were not detected.

Due to the high morphological and genetic variance in the *Aurelia aurita* species also variations in the physiological and toxinological characteristics are likely (Dawson & Martin, 2001; Dawson, 2003). Radwan et al., (2001) compared animals from the Red Sea and Chesapeake Bay and could show increased haemolytic, dermonecrotic and phospholipase activities in organisms originating from the Red Sea. An indication for such regional differences has been shown for different regions of the North Sea in the present publication. The more toxic organisms from the Western Isles were collected in a bay with aquaculture facilities whereas the sampling area at the Orkney Islands was a relatively undisturbed region. However, it can not be precluded that the differences in the toxic potential reflects an adaptation to the eutrophication and nutrition conditions in these two different spots.

The significant predation impact of *Cyanea capillata* on zooplankton organisms especially fish larvae, could be shown in field observations and laboratory studies (Purcell, 2003; Hay et al., 1990; Fancett & Jenkins, 1988; Brewer, 1989). Since the scyphozoa *C. capillata* express a higher envenomation capacity, a few *in vivo* and *in vitro* studies concerning its

toxinological potency and biochemical composition of the venom have been performed however the toxic effect on cultivated gill cells have not been shown before. Detailed pharmacological studies can be found in Walker, (1977) where a cardiotoxic activity is related to a 70 kDa protein. A hemolytic activity is related to proteins smaller than 10 kDa and no haemolytic activity was found in organism smaller than 15 cm (Long & Burnett, 1989). Since no animals smaller than 20 cm have been tested in the present investigation, this size dependency could not be confirmed. Previously, in vitro experiments with hepatocytes as target showed a cytotoxic effect above protein equivalent concentrations of 7 µg mL^{-1} indicating the increased susceptibility of fish gill cells against *Cyanea* venoms compared to mammalian cells (Helmholz et al., 2007).

Rice and Powell tested the ability of tentacle venom of isolated nematocyst to induce dermal reactions and found only mild erythema in a few cases. The proteinaceous nature and the nematocyst origin of the active substance were pointed out (Rice & Powell, 1972). The reaction of dermal skin and the nematocyst discharge was also observed by (Heeger et al., 1992) but in both studies no information on the biochemical composition of the venom can be found.

Since only a few toxins have been isolated from jellyfish, an interspecies comparison of the detected proteins is difficult. Lethal toxins from box jellyfish species *Carybdea alata* and *Carybdea rastoni* with a molecular weight of 43, 45, and 46 kDa, respectively have been isolated and sequenced (Brinkman & Burnell, 2007; Nagai, 2003). A remarkable cluster of protein bands in same molecular weight range could be detected in the venom of both investigated northern scyphozoan species making this region especially interesting for further structural analytical investigations.

Conclusion and outlook

The application of the a rainbow trout gill cell line for the detection of the toxic potential of whole venoms derived from purified cnidocyst suspensions showed a strong susceptibility of these cells. Morphological changes in cell growth can be observed within one hour after venom exposure and a fluorescence-based quantification of the cell viability shows a measurable dose-dependent effect at protein equivalent concentration above 1 µg mL^{-1}. The comparatively high susceptibility of the gill cells enables the analysis of a number of samples requiring low amounts of venoms. This *in vitro* approach provides a stable and reproducible in vitro test system for the detection and comparison of the ichthyotoxic potential of cnidarian species. Comparing the two dominant northern species,

the increased toxic potency of *C. capillata* compared to *A. aurita* could be shown. The toxic potential is dependent on organismal size. The venom obtained from cnidocysts of oral arms is more potent than that obtained from fishing tentacles. This could possibly be related to the occurrence of proteolytic enzymes function as digestion enzymes and affecting the gill cells unspecifically. Whereas the more neuroactive toxins inducing the paralysis of prey organisms are present in the fishing tentacles (Lassen 2009 this issue). Biochemical analysis of whole venoms utilizing gel electrophoresis showed a high venom complexity with a broad molecular range of detected proteins. Similarities within isolated toxins from box jellyfish, described in the literature, could be detected. Differences in the venoms of the two species from different regions and their mesenteric and fishing tentacles could be shown but further detailed structural analysis have to be performed in order to reveal structure-effect relationships and quantitative evidences.

The next step after establishing the gill toxicity assay is the parallel application of *in vivo* laboratory experiments to correlate the *in vitro* toxic potential with data on the quantitative impact on prey organisms in order to evaluate the relevance and applicability of *in vitro* cell-based assays for ecological questions critically.

Acknowledgements

The authors would like to thank Dr. Christian Schuett and colleagues from the Alfred-Wegener-Institute Station Helgoland and the crew of the RV Heincke for their substantial help and support and Dr. Juergen Gandrass (Institute for Coastal Research, Department for Marine Bioanalytical Chemistry) for critically reading the manuscript.

References

1. Bailey, K. M. & R. S. Batty, 1984. Laboratory study of predation by *Aurelia aurita* on larvae of cod, flounder, plaice and herring: development and vulnerability to capture. Marine Biology 83: 287-291.
2. Bamstedt, U., 1990. Trophodynamics of the scyphomedusae *Aurelia aurita* – predation rates in relation to abundance, size and type of prey organism. Journal of Plankton Research 12: 215-229.
3. Bamstedt, U. & M. B. Martinussen, 2000. Estimating digestion rate and the problem of individual variability, exemplified by a scyphozoan jellyfish. Journal of Experimental Marine Biology and Ecology 251: 1-15.
4. Bamstedt, U., M. B. Martinussen, & S. Matsakis, 1994. Trophodynamics of the two scyphozoan jellyfishes, *Aurelia aurita* and *Cyanea capillata*, in western Norway. ICES Journal of Marine Science 51: 369-382.
5. Barz, K. & H.-J. Hirche, 2007. Abundance, distribution and prey composition of scyphomedusae in the southern North Sea. Marine Biology 151: 1021-1033.

6. Behrends, G. & G. Schneider, 1995. Impact of *Aurelia aurita* medusae (Cnidaria, Scyphozoa) on the standing stock and community composition of mesozooplankton in the Kiel Bight (western Baltic Sea). Marine Ecology Progress Series 127: 39-45.
7. Blum, H., H. Beier, & H.J. Gross, 1987. Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gels. Electrophoresis 8: 93-99.
8. Bols, N., A. Barlian, M. Chirino-Trejo, S.J. Caldwell, P. Geogan, & L.E.J. Lee, 1994. Development of a cell line from primary cultures of rainbow trout, *Oncorhynchus mykiss* (Walbaum), gills. Journal of Fish Diseases 17: 601-611.
9. Bradford, M. 1976. A rapid and sensitive method for the quantitation of microgramm quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72: 248-254.
10. Brewer, R. H. 1989. The annual pattern of feeding, growth, and sexual reproduction in *Cyanea* (Cnidaria: Scyphozoa) in the Niantic River Estuary, Connecticut. Biological Bulletin 176: 272-281.
11. Brinkman, D. & J. Burnell, 2007. Identification, cloning and sequencing of two major venom proteins from the box jellyfish, *Chironex fleckeri*. Toxicon 50: 850-860.
12. Burnett, J. W., G. Calton & J.B. Larsen, 1988. Significant envenomation by *Aurelia aurita*, the moon jellyfish. Toxicon 26: 215-217.
13. Dawson, M. N., 2003. Macro-morphological variation among cryptic species of the moon jellyfish, *Aurelia* (Cnidaria: Scyphozoa). Marine Biology 143: 369-379.
14. Dawson, M. N. & L. E. Martin, 2001. Geographic variation and ecological adaptation in *Aurelia* (Scyphozoa, Semaeostomeae): some implications from molecular phylogenetics. Hydrobiologia 451: 259-273.
15. Dayeh, V. R., D. H. Lynn, & N.C. Bols, 2005. Cytotoxicity of metals common in mining effluent to rainbow trout cell lines and to the ciliated protozoan, *Tetrahymena thermophila*. Toxicology in vitro 19: 399-410.
16. Dayeh, V. R., K. Schirmer & N.C. Bols, 2002. Applying whole-water samples directly to fish cell cultures in order to evaluate the toxicity of industrial effluent. Water Research 36: 3727-3738.
17. Fancett, M. S. & G. P. Jenkins, 1988. Predatory impact of scyphomedusae on ichthyoplankton and other zooplankton in Port Phillip Bay. Journal of Experimental Marine Biology and Ecology 116: 63-77.
18. Fenner, P. & P. Fitzpatrick, 1986. Experiments with the nematocysts of *Cyanea capillata*. The Medical Journal of Australia 145: 174.
19. Gröndahl, F., 1988. A comparative ecological study on the scyphozoans *Aurelia aurita, Cyanea capillata* and *Cyanea lamarckii* in the Gullmar Fjord, Western Sweden, 1982 to 1986. Marine Biology 97: 541-550.
20. Hansson, L. J., 2006. A method for in situ estimation of prey selectivity and predation rate in large plankton, exemplified with the jellyfish *Aurelia aurita* (L.). Journal of Experimental Marine Biology and Ecology 328: 113-126.
21. Hay, S. (2006). Marine ecology: Gelatinous bells may ring change in marine ecosystems. Current Biology 16: R679-R682.
22. Hay, S., J. Hislop & A.M. Shanks, 1990. North Sea Scyphomedusae; summer distribution, estimated biomass and significance particularly for 0-group gadoid fish. Netherlands Journal of Sea Research 25: 113-130.
23. Heeger, T., H. Möller & U. Mrowietz, 1992. Protection of human skin against jellyfish (*Cyanea capillata*) stings. Marine Biology 113: 669-678.

24. Helmholz, H., C. Ruhnau, C. Schütt & A. Prange, 2007. Comparative study on the cell toxicity and enzymatic activity of two northern scyphozoan species *Cyanea capillata* (L.) and *Cyanea lamarckii* (Peron & Leslueur). Toxicon 50: 53-64.
25. Hiromi, J., s. Handa & T. Sekine, 1997. Lethal effect of an autolysate of *Aurelia aurita* on red tide flagellates. Fisheries Sciences 63: 478-479.
26. Kihara, H., M. Anraku & O. Hirokazu, 1988. Tetrodotoxin-uneffected depolarization of frog muscles induced by the venom of jellyfish (Genus: *Aurelia*). Japanese Journal of Physiology 38: 839-849.
27. Kintner, A. H., J. E. Seymour & S.L. Edwards, 2005. Variation in lethality and effects of two Australian chirodropid jellyfish venoms in fish. Toxicon 46: 699-708.
28. Lee, L. E. J., K. Schirmer & N.C. Bols, 2008. Applications and potential uses of RTgill-W1, a cell line derived from gills of rainbow trout. In Vitro Cellular & Developmental Biology-Animal 44: 17-17.
29. Long, K. O. & J. W. Burnett, 1989. Isolation, characterization, and comparison of hemolytic peptides in nematocyst venoms of two species of jellyfish (*Chrysaora quinquecirrha* and *Cyanea capillata*). Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 94: 641-646.
30. Lynam, C. P., M. J. Gibbons, B.E. Axelsen, C.A.J. Sparks, J. Coetzee, B.G. Heywood & A.S: Brierley, 2006. Jellyfish overtake fish in a heavily fished ecosystem. Current Biology 16: 1976.
31. Lynam, C. P., M. R. Heath, S.J. Hay & A.S. Brierley, 2005. Evidence for impacts by jellyfish on North Sea herring recruitment. Marine Ecology-Progress Series 298: 157-167.
32. Malej, A., V. Turk, D. Lučić & A. Benović, 2007. Direct and indirect trophic interactions of *Aurelia* sp. (Scyphozoa) in a stratified marine environment (Mljet Lakes, Adriatic Sea). Marine Biology 151: 827-841.
33. Martinussen, M. B. & U. Bamstedt, 2001. Digestion rate in relation to temperature of two gelatinous planktonic predators. Sarsia 86: 21-35.
34. Mills, C. 2001. Jellyfish blooms: are populations increasing globally in response to changing ocean conditions. Hydrobiologia 451: 55-68.
35. Möller, L. F. & H. U. Riisgaard, 2007. Population dynamics, growth and predation impact of the common jellyfish *Aurelia aurita* and two hydromedusae, *Sarsia tubulosa* and *Aequorea vitrina*, in Limfjorden (Denmark). Marine Ecology Progress Series 346: 153-165.
36. Möller, H. 1980. Scyphomedusae as predators and food competitors of larval fish. Meeresforschung 28: 90-100.
37. Nagai, H. 2003. Recent progress in jellyfish toxin study. Journal of Health Science 49: 337-340.
38. Omori, M., H. Ishii & A.I: Fujinaga, 1995. Life history strategy of *Aurelia aurita* (Cnidaria, Scyphomedusae) and its impact on the zooplankton community of Tokyo Bay. ICES Journal of Marine Sciences 52: 597-603.
39. Purcell, J. E., 2003. Predation on zooplankton by large jellyfish, *Aurelia labiata*, *Cyanea capillata* and *Aequorea aequorea*, in Prince William Sound, Alaska. Marine Ecology-Progress Series 246: 137-152.
40. Purcell, J. E., S. Uye & W.T: Lo, 2007. Anthropogenic causes of jellyfish blooms and their direct consequences for humans: a review. Marine Ecology-Progress Series 350: 153-174.

41. Radwan, F. F. Y., J. W. Burnett, D. A. Bloom, T. Coliano, M. E. Eldefrawi, H. Erderly, L. Aurelian, M. Torres & E. P. Heimer-de la Cotera, 2001. A comparison of the toxinological characteristics of two *Cassiopea* and *Aurelia* species. Toxicon 39: 245-257.
42. Regula, C., S. P. Colin, J.H. Costello & H. Kordula, (2009). Prey selection mechanism of ambush-foraging hydromedusae. Marine Ecology Progress Series 374: 135-144.
43. Rice, N. & W. Powell (1972). Observations on three species of jellyfish from Chesapeake bay with special reference to their toxins. II *Cyanea capillata*. Biological Bulletin 143: 617-622.
44. Schaegger, H. & G. von Jagow 1987. Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. Analytical Biochemistry 166: 368-379.
45. Schirmer, K., D. G. Dixon, B.M. Greenberg, D.G. Dixon & N. C. Bols, (1998). Ability of 16 priority PAHs to be directly cytotoxic to a cell line from the rainbow trout gill. Toxicology 127: 129-141.
46. Segura-Puertas, L., G. Avila-Soria, J. Sanchez-Rodgriguez, M. E: Ramos-Aguilar & J.W. Burnett, (2002). Some toxinological aspects of *Aurelia aurita* (Linne) from the Mexican Caribbean. Journal of Venomous Animals and Toxins 8: 113-128.
47. Suchman, C. L. & B. K. Sullivan 2000. Effect of prey size on vulnerability of copepods to predation by the scyphomedusae *Aurelia aurita* and *Cyanea* sp. Journal of Plankton Research 22: 2289-2306.
48. Sullivan, B. K., C. L. Suchman & J.H. Costello, J. H. 1997. Mechanics of prey selection by ephyrae of the scyphomedusa *Aurelia aurita*. Marine Biology 130: 213-222.
49. Titelman, J., L. Gandon, A. Goarant & T. Nilsen, 2007. Intraguild predatory interactions between the jellyfish *Cyanea capillata* and *Aurelia aurita*. Marine Biology 152: 745-756.
50. Walker, M. J. A. (1977a). The cardiac actions of a toxin-containing material from the jellyfish, *Cyanea capillata*. Toxicon 15: 15-27.
51. Walker, M. J. A. (1977b). Pharmacological and biochemical properties of a toxin containing material from the jellyfish, *Cyanea capillata*. Toxicon 15: 3-14.
52. Walker, M. J. A., T. T. Martinez & D.V. Godin, 1977. Investigations into the cardiotoxicity of a toxin from the nematocysts of the jellyfish, *Cyanea capillata*. Toxicon 15: 339-346.
53. Wiersbitzky, S., G. Hegewald & B. Gibb 1973. Glomerulo-tubular nephrosis in rabbits and injection of aqueous extracts from jellyfish (*Aurelia aurita*)." Experimental Pathology 8: 232-235.

List of tables and figures

Table 1 Samples *Aurelia aurita*

Sample	Average umbrella diameter (cm) *	Region/Year
A. aur 1	8.2 ± 0.9 (*n* = 66)	Lewis/West Coast Western Isles/2007
A. aur 2	13.1 ± 1.9 (*n* = 888)	Lewis/West Coast Western Isles/2007
A. aur 3	23.4 ± 3.5 (*n* = 651)	Lewis/West Coast Western Isles/2008
A. aur 4	32.1 ± 1.9 (*n* = 82)	Lewis/West Coast Western Isles/2008
A. aur 5	9.1 ± 0.9 (*n* = 81)	Mainland/ Orkney Islands/2008
A. aur 6	12.0 ± 1.2 (*n* = 90)	Mainland/ Orkney Islands/2008

* Mean ± standard deviation; *n* = number of individuals

Table 2 Samples *Cyanea capillata* and cell viability after exposure to mesenteric tentacle venom

Sample	Average umbrella diameter (cm) *	Region/Year	Cell viability MT venom (%) **
C. cap 1	21.5 ± 6.0 (*n* = 23)	Rousay/ Orkney Islands/2006	7.42 (4.06)
C. cap 2	33.0 ± 0.9 (*n* = 6)	Rousay/ Orkney Islands/2006	16.32 (7.54)
C. cap 3	20.7 ± 5.5 (*n* = 32)	Priwall/ Baltic Sea/2006	36.40 (10.87)
C. cap 4	20.9 ± 4.5 (*n* = 72)	Eckernfoerde/ Baltic Sea/2008	29.13 (9.75)
C. cap 5	33.0 ± 2.8 (*n* = 2)	Eckernfoerde/ Baltic Sea/2008	32.05 (10.32)

* Mean ± Standard deviation; *n* = number of individuals

** Venom mesenteric tentacle (MT) concentration 2 µg mL^{-1};

Mean (standard deviation); *n* > 10

Figure 1:
Continuous culture of RTgill-W1 cells growing in a monolayer on cell culture flask surface (A) cultivation 8 days (B) cultivation 14 days

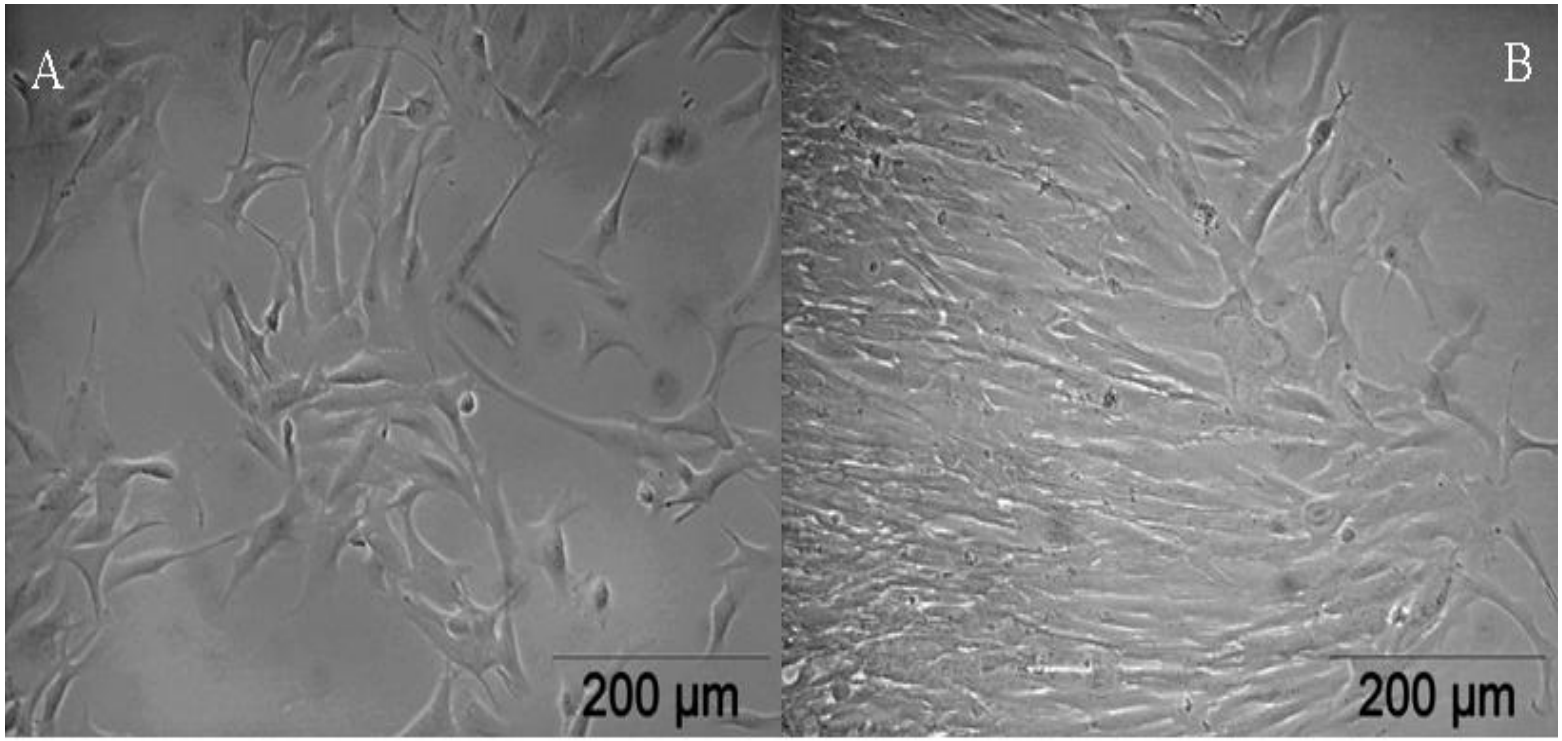

Figure 2:
Influence of increasing dosages of *Aurelia aurita* venom on RTgill-W1 cell growth (A) positive control (B) 5 µg mL^{-1} (C) 20 µg mL^{-1} (D) 50 µg mL^{-1}

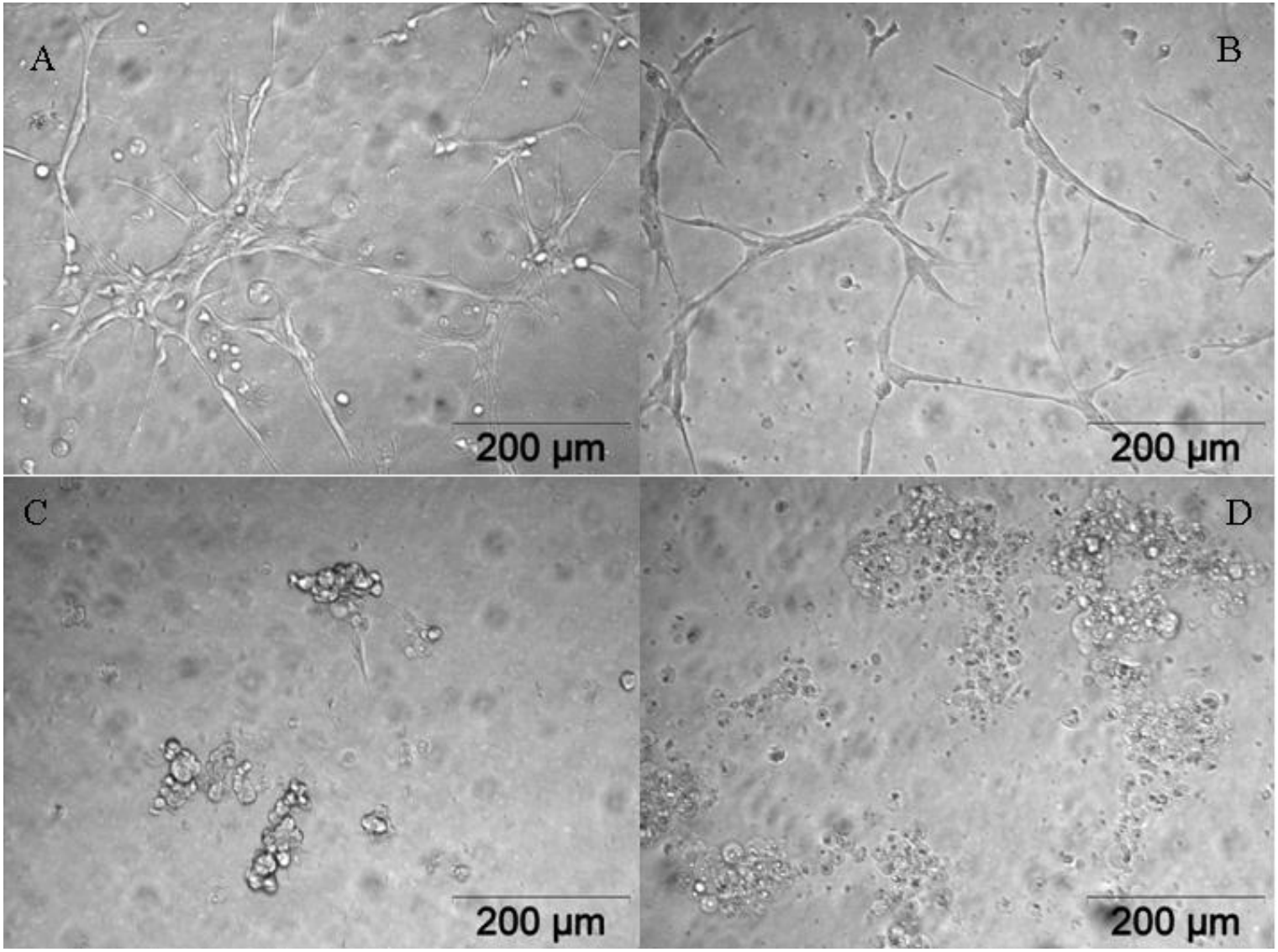

Figure 3:
Dose dependent cytotoxic effect on cell viability of whole venom of *Aurelia aurita* (sample A. aur 2 umbrella diameter > 10 cm; Western Isles) mesenteric (MT) and fishing (FT) tentacle (M ± SD; $n > 9$)

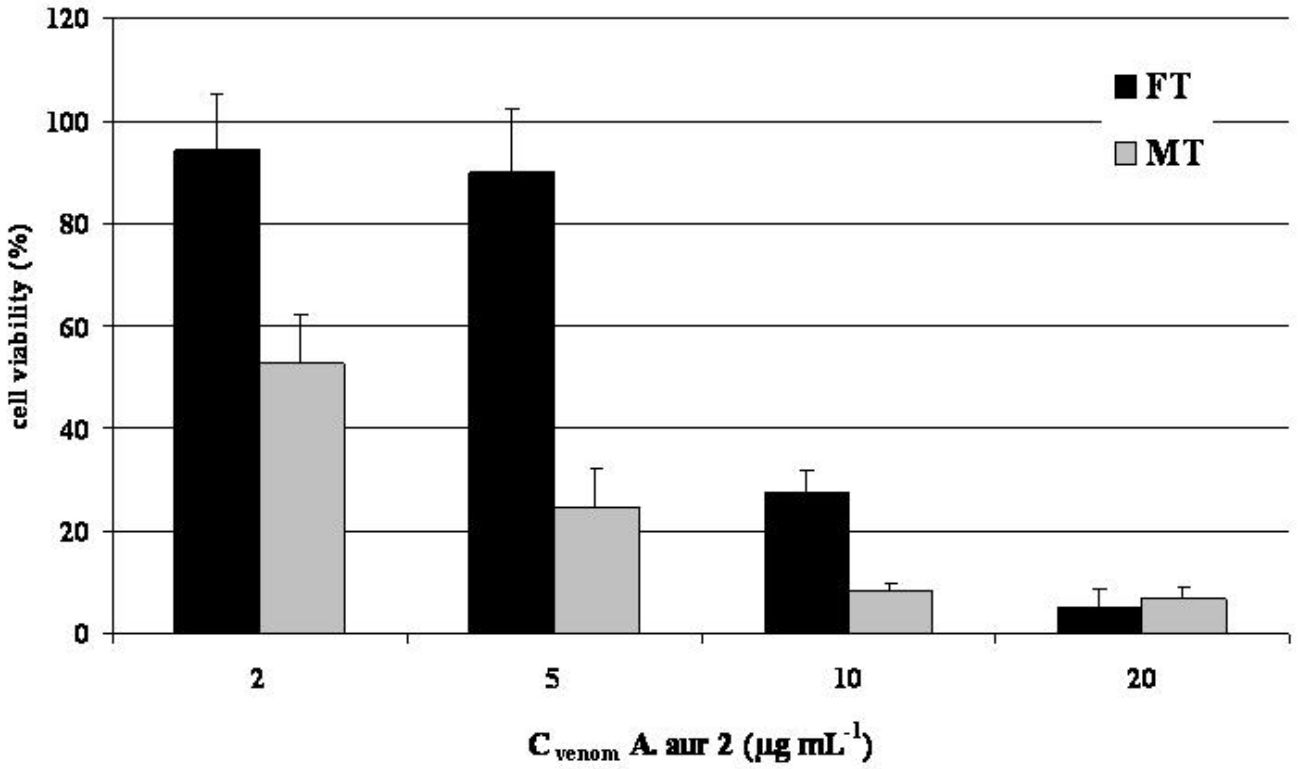

Figure 4:
Gill cell toxicity of fishing tentacle venom of *Aurelia aurita* (M ± SD $n > 10$; *$P > 0.05$ Mann – Whitney Test)

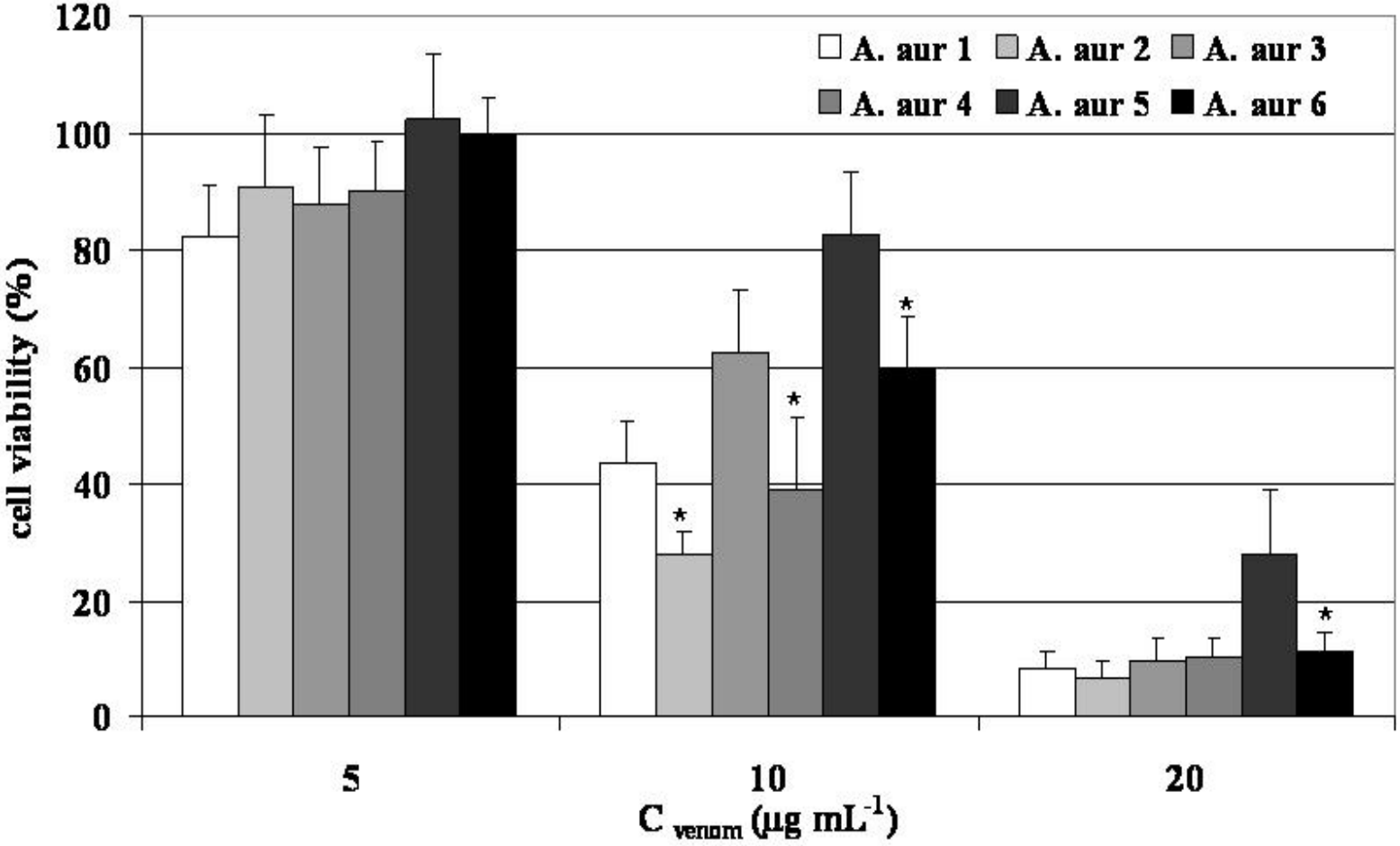

Figure 5:
(A) SDS Page of crude venoms (protein equivalents 2 µg) of fishing (FT) and mesenteric (MT) tentacle of *Aurelia aurita*; M – Marker (numbers indicate the molecular weight in kDa) Lane 1: A. aur 1 FT; Lane 2: A. aur 2 FT; Lane 3: A. aur 3 FT; Lane 4: A. aur 4 FT; Lane 5: A. aur 5 FT; Lane 6: A. aur 6 FT; Lane 7: A. aur 1 MT; Lane 8: A. aur 2 MT; Lane 9: A. aur 3 MT; Lane 10: A. aur 4 MT; Lane 11: A. aur 5 MT; Lane 12: A. aur 6 MT

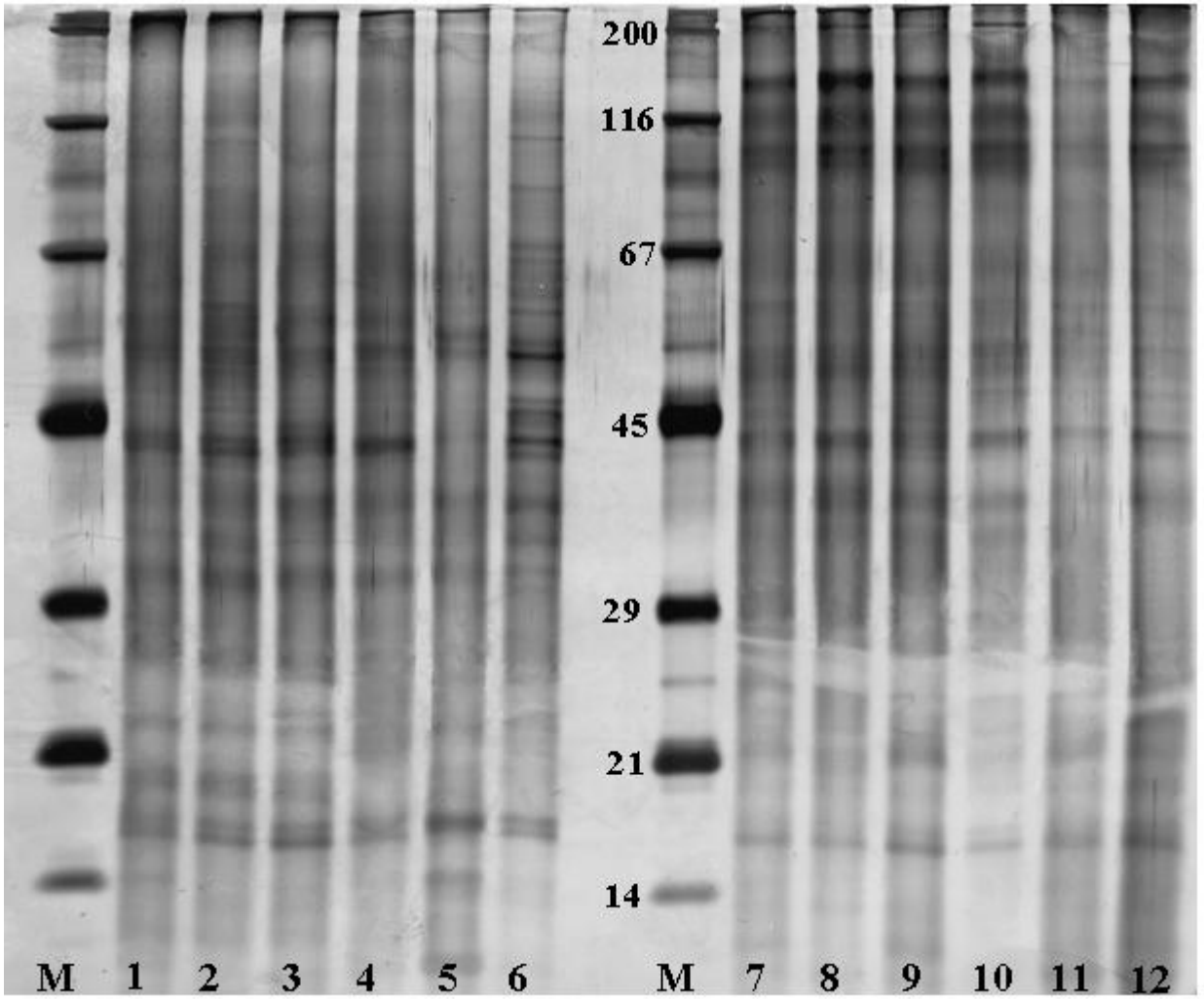

Figure 5:
(B) SDS Page of crude venoms (protein equivalents 2 µg) of fishing (FT) and mesenteric MMT) tentacle of *Cyanea capillata*; M – Marker (numbers indicate the molecular weight in kDa) Lane 1: C. cap 1 FT; Lane 2: C. cap 2 FT; Lane 3: C. cap 3 FT; Lane 4: C. cap 4 FT; Lane 5: C. cap 5 FT; Lane 6: C. cap 1 MT; Lane 7: C. cap 2 MT; Lane 8: C. cap 3 MT; Lane 9: C. cap 4 MT; Lane 10: C. cap 5 MT