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Marine Invertebrate Cell Cultures: New Millennium Trends

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Abstract

This review analyzes activities in the field of marine invertebrate cell culture during the years 1999 to 2004 and compares the outcomes with those of the preceding decade (1988 to 1998). During the last 5 years, 90 reports of primary cell culture studies of marine organisms belonging to only 6 taxa (Porifera, Cnidaria, Crustacea, Mollusca, Echinodermata, and Urochordata) have been published. This figure represents a 2-fold increase in the annual number of publications over the decade 1988 to 1998. Three other trends distinguish the two reviewed periods. First, in recent years studies attempting to improve cell culture methodologies have decreased, while interest in applications of already existing methodologies has increased. This reflects the effects of short-term cultures in attracting new researchers and scientific disciplines to the field. Second, only 17.8% of the recent publications used long-term cultures, compared with 30.0% of the publications in the previous decade. Third, during recent years research in cell cultures has studied fewer model species more extensively (mainly, *Botryllus schlosseri, Crassostrea, Mytilus, Penaeus, and Suberites domuncula*), signifying a shift from previous investigations that had studied a more diverse range of organisms. From 1988 to 1998 the phylum Mollusca was the most studied taxon (34.4%), but recent years have seen more studies of Porifera and Crustacea (30.0% and 32.2% of publications) than of Mollusca (21.1%). Still, not even a single established cell line from any marine invertebrate has yet been made available. However, the use of new cellular, genomic, and proteomic tools may fundamentally change our strategy for the development of cell cultures from marine invertebrates.

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Introduction

Cells from vertebrates, insects, arachnids, and plants, under in vitro conditions, are being used in a variety of circumstances and in many scientific disciplines as exceptionally important tools for experimentation. Yet, for reasons that remain obscure, all endeavors to develop cell cultures from marine invertebrates have been ineffective so far (Rinkevich et al., 1994; Bayne, 1998; Rinkevich, 1999; Mothersill and Austin, 2000) despite the acknowledged need for cell cultures from species that are important in aquaculture or in the pharmaceutical industry. Attempts to develop cell cultures from many marine invertebrate taxa date back about a century (Gomot, 1971; Rannou, 1971; literature cited in Mothersill, and Austin, 2000). This is a confounding outcome since many cell types from a variety of marine invertebrates have wide morphogenetic potentialities (multipotency, totipotency, including neoplasia; Rinkevich et al., 1994; Rosenfield et al., 1994). The above phenomenon leads to high in vivo plasticity of shapes, structures, cell replacement mechanisms, proliferation processes, and cell lineages, in different invertebrate taxa. The high plasticity is also reported between systematically related groups of organisms (Rinkevich, 1999).

The last 2 decades have witnessed a variety activities, by numerous laboratories, to develop cell cultures from marine organisms (Bayne, 1998; Rinkevich, 1999; Mothersill and Austin, 2000). However, limited activities in marine invertebrate cell cultures have been reported (Rosenfield, 1993), mostly because scientific journals usually do not publish failed experiments (Rinkevich, 1999). Within this context of limited publication exposure, a

previous review on marine invertebrate cell culture activities from 1988 to 1998 (Rinkevich, 1999) depicted 90 peer-reviewed publications. Only 27 (30%) of the publications in this list described cases in which cultures were maintained in vitro for more than one month. In 4 phyla/classes (Cnidaria, Crustacea, Mollusca, and Echinodermata, approximately 29% of the publications described long-term studies, but in the Porifera and Urochordata, these numbers rose to 36.4% and 45.5%, respectively. However, in 18 of these long-term reports, it was suspected or confirmed that the developed cultures were contaminated by alien prokaryotic cells. Again, these attempts failed to yield a single valid invertebrate cell culture, although it has been confirmed that all cells of different taxa within the kingdom Animalia are basically the same in that they have similar nutrient requirements, are controlled by the same developmental and physiologic-biochemical pathways, and are under the expression of identical genes (Rinkevich, 1999).

Reviews on marine invertebrate cell cultures (Bayne, 1998; Rinkevich, 1999; Mothersill and Austin, 2000) have pointed further to several topics that emerged in the last few years. These topics include the development of unique fundamental techniques (such as methodologies and specific culture media), the choice of cell, tissue, organ, and organism source for the study, the use of tissue fragments and nonadherent cells, the importance of cell characterizations under in vitro conditions, the validation of in vitro techniques, and the need for cryopreservation techniques for cells.

The increasing need for cell cultures from marine invertebrates in the last several years emanates from two major economic trends; (1) the demand for commercial supply of novel biologically active chemical compounds with pharmaceutical potential; and (2) the need for scientific tools to study animal endocrinology and diseases of edible species (crayfish, lobsters, shrimps, mussels, oysters, and clams). The continued failure to establish cell cultures from marine invertebrate taxa may therefore suggest that we still lack vital information regarding invertebrates' cell requirements and their physiology and biochemical patterns in toto and in vitro. During the period covered by the last review on marine invertebrate cell cultures (Rinkevich, 1999), it had been suggested that efforts should concentrate on such approaches as transgenesis (Rosenfield et al., 1994), mutagenesis by irradiation and chemicals, cell hybridization (Diekmann-Schuppert et al., 1989), replacement of vertebrate sera with lipids and other factors (Goodwin, 1991), and the use of feeder layers.

This review seeks to summarize the attempts to obtain cell cultures from marine invertebrates made within the 5 years (1999–2004) since the lost review (Rinkevich, 1999). Special attention was given to the trends shaping this scientific discipline toward future accomplishments.

Activities During 1999 to 2004

As in the period covered by the previous review (1988–1998; Rinkevich, 1999), the last 5-year period (1999–2004) has witnessed attempts to develop cell cultures from marine invertebrates concentrating on a few species, within 6 exclusive taxa (Porifera, Cnidaria, Crustacea, Mollusca, Echinodermata, and Urochordata) out of more than 20 invertebrate phyla available (Fig. 1). As before (Rinkevich, 1999), within each studied phylum only a limited number of scientific groups and species were studied for cell cultures. For example, not even a single study was performed on the phyla Annelida and Platyhelminthes (as compared with a single report on marine annelid cell culture and a single cell culture from a freshwater planarian during 1988–1998; Rinkevich, 1999), only a few groups of decapods were studied in the crustaceans, not a single marine hydrozoan or schyphozoan in the Cnidaria was studied, no cephalopods, and no opistobranchia in the mollusks, and so forth. Again, this emphasizes (Rinkevich, 1999) the remarkable lack of diversity in the studies for developing cell cultures from marine invertebrates.

In total 90 original publications were carefully evaluated (Fig. 1), revealing a substantial demand for cell cultures from marine invertebrates. Three classes of publications were discerned: those that concentrated on the development of cell cultures, studies that attempted to use already existing cell culture methodologies for applied aspects, and review articles. A short description of the last 5 years of activities in marine invertebrate cell cultures is presented below for each phylum/taxon.

Porifera

While during the preceding 10 years (1988–1998; Rinkevich, 1999) only 11 studies on sponge tissue cultures were published, during the next 5 years (1999–2004) a total of 27 publications appeared, reflecting a nearly 5-fold increase (Fig. 2). However, only 12 publications (Fig. 1; 44.4%; 3 of the publications are reviews; 2 in Mothersill and Austin, 2000) were directly related to the establishment of cell cultures from sponges (Müller et al., 1999; Willoughly and Pomponi, 2000; De Rosa et al., 2001,

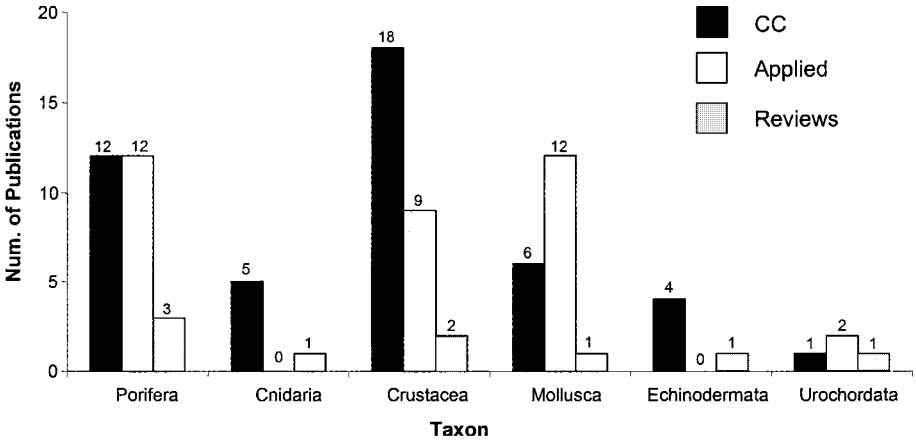

Fig. 1. Marine invertebrate cell culture publications from 1999 to 2004 (sorted from ASFA, MEDLINE, and BIOSIS). Numbers of cell culture publications (black bars, CC), applied research studies (white bars), and reviews (shaded bars) for 6 taxa studied.

2002, 2003; Nickel et al., 2001; Krasko et al., 2002; Richelle-Maurer et al., 2003; Sipkema et al., 2003a,b; Zhang W et al., 2003, Zhang X et al., 2003, 2004). Six of these 12 publications (Müller et al., 1999; Nickel et al., 2001; Krasko et al., 2002; De Rosa et al., 2003; Sipkema et al., 2003b; Zhan X et al., 2004) used, at least in part of their experiments, the Mediterranean species *Suberites domuncula*, and only 4 publications (Müller et al., 1999; Nickel et al., 2001; Sipkema et al., 2003b; Zhang X et al., 2003) dealt with >1-month-old cultures. Five of the publications (Willoughly and Pomponi, 2000; De Rosa et al., 2002, 2003; Richelle-Maurer et al., 2003) used cell cultures in suspensions, while the other 6 studies used cell aggregates. All 12 studies were at the stage of primary cultures without any significant breakthrough in the development of long-lasting cell cultures. It was also concluded (Sipkema et al., 2003b) that the commonly used cell aggregate systems may be very suitable to start in vitro sponge cultures, as they can be produced from many different sponges, which circumvents problems associated with transport of living sponges. Cell aggregate processes may also serve as purification steps of the dissociated sponge-cell suspension from fungi and nonsymbiotic bacteria. Redissociation of cell aggregates into single cells could thus lead to an axenic sponge-cell suspension and perhaps facilitate research on sponge-cell cultures.

During 1999 to 2004, none of the published studies on sponges demonstrated a substantial increase in cell numbers or biomass due to real cell proliferation. Yet much attention was given to the application of the already established primary cultures (Andrade et al., 1999; Krasko et al., 1999; Müller et al., 2000; Osinga et al., 2001; De Rosa et al., 2002; Lopez et al., 2002; Kelve et al., 2003; Sipkema et al., 2003a,b, 2004; Zhao et al., 2003; Mitova et al., 2004; Custodio et al., 2004). The literature reveals that cell culture methodologies have not changed significantly during the period under review. Research efforts inclined, therefore, toward the use of the primary cultures for applied purposes, elucidating the expression of genes or biosynthesis of natural product (Andrade et al., 1999; Krasko et al., 1999; Müller et al., 2000; De Rosa et al., 2002; Kelve et al., 2003), revealing some biological characteristics such as sponge-microbe associations (Osinga et al., 2001; Mitova et al., 2004; Sipkema et al., 2004), molecular identification of primary cultures (Lopez et al., 2002; Sipkema et al., 2003a), allorecognition pathways (Custodio et al., 2004), and cellular aspects of cell cultures (Zhao et al., 2003; Sipkema et al., 2004). As with cell culture studies, 4 applied publications (Krasko et al., 1999; Osinga et al., 2001; Mitova et al., 2004; Sipkema et al., 2004) dealt with *Suberites domuncula*, and 2 publications (Müller et al., 2000; Sipkema et al., 2003b) studied *Dysidea avara*.

The aforementioned studies provided a new insight into the ill-defined field of sponge-associated microsymbionts. The high diversity of these consortia (Lopez et al., 2002; Osinga et al., 2001) probably has significant economic importance, as at least some of the novel natural products isolated from sponges are produced by the sponge-associated organisms (Faulkner et al., 1999). As such, the development of molecular markers (such as AFLP, ITS, 18S rDNA sequences, and others; Lopez et al., 2002; Sipkema et al., 2003a) is of primary importance for the evaluation of the roles of sponge endosymbionts in sponge biology and biotechnological approaches.

Cnidaria

Five studies relating to the development or improvement of cell cultures from corals (Frank and

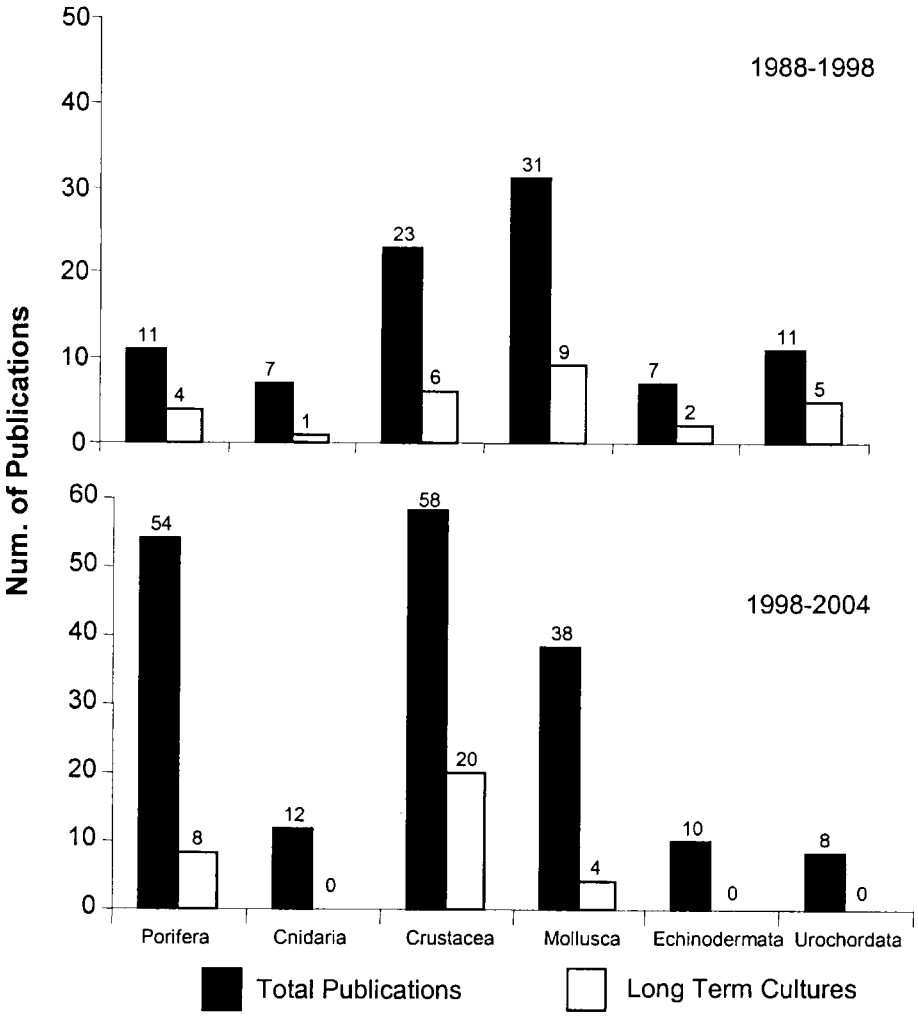

Fig. 2. Marine invertebrate cell culture publications from 1988 to 1998 and from 1999 to 2004 (numbers doubled for comparison with a period of a decade), sorted from ASFA, MEDLINE, and BIOSIS. Total of publications (black bars) and cultures longer than 1 month (white bars) for 6 taxa studied.

Rinkevich, 1999; Kopecky and Ostrander, 1999; Schmid et al., 1999; Domart-Coulon et al., 2001, 2004) have been published during the last 5 years, in addition to a single review on the application of cell cultures for coral-reef ecology (in Mothersill and Austin, 2000; Fig. 1). This represents an increase in scientific products as compared to the 7 related publications in the entire preceding decade (Rinkevich, 1999; Fig. 2).

All of the above studies represented short-term experiments (<1 month), however, with significant implications for holding cnidarian cells in vitro. While the phylum Cnidaria is known to be highly morphologically diverse, it is probably also molecularly variable. This was made evident by studies revealing specificity of cell-substrate adhesion (Frank and Rinkevich, 1999; Schmid et al., 1999) and aragonite crystallization (Domart-Coulon et al., 2001). It was also concluded that in cnidarians the role of cell-substrate interactions in maintaining the differentiated state of tissues, cell migration, differentiation, and morphogenesis in general (Frank and Rinkevich 1999) is the same as in other phyla, including the vertebrates. This reflects also adherent primary cell cultures in which enhanced alkaline phosphatase activity was followed by the precipitation of aragonite crystals. Although some encouraging reports on cell culture systems were published during this period (Kopecky and Ostrander, 1999; Domart-Coulon et al., 2004), none (as in earlier studies, Rinkevich, 1999) demonstrated convincingly that cnidarian cells propagate and survive for a prolonged time under culture conditions.

Crustacea

In total 29 new publications pertaining to tissue cultures in Crustacea (including a single report dealing with horseshoe crab; Joshi et al., 2002) were published during the last 5 years (Fig. 1), a figure reflecting more than double the previous publication rate of 23 manuscripts in the preceding decade

(Rinkevich, 1999; Fig. 2). Out of these studies, 18 (62.1%) dealt directly with tissue culture, 9 (31%) delivered the applied approach by the use of the available current knowledge, and 2 publications were review articles (Fig. 1).

The 18 tissue culture studies (Braasch et al., 1999; Fraser and Hall, 1999; Ganter et al., 1999; Itami et al., 1999; Kasornchandra et al., 1999; Owens and Smith, 1999; Walton and Smith, 1999; West et al., 1999; Mulford et al., 2001; Shimizu et al., 2001; Wang et al., 2001; Joshi et al., 2002; Lang et al., 2002a, 2002b; Gao et al., 2003; Maeda et al., 2003; Lang et al., 2004; Stephanyan et al., 2004) comprised 10 publications dealing with long-term cultures (> month). Two studies demonstrated exceptionally long culture periods: primary cultures derived from ovarian tissue of the shrimp *Penaeus monodon* revealed 4 morphologically different types of ovarian cells (epithelioid, fibroblastic, rounded, and epithelioid with large nuclei) that were maintained for up to 17 months. Epithelioid cells grew best in modified Grace's medium but were generally short living (less than 2 months). In this study fibroblast-like cells that formed confluent monolayers in modified 2× L-15 medium were passed thrice and survived for 17 months (Fraser and Hall, 1999). From the same species Owens and Smith (1999) maintained heart cells in vitro for more than 10 months. These cultures exhibited cells dividing for up to 40 days after isolation. Fourteen (79%) of the aforementioned 18 studies were performed on shrimps, mainly on different species of the genus *Penaeus* (10 studies). These studies concentrated on *Penaeus* hepatopancreatic cells, ovarian tissue, cells from lymphoid organ tissues, heart cells, nerve cord cells, and hematopoietic cells. For developing continuous in vitro cell replication from *Penaeus*, cell cycle regulatory proteins (cyclins) were identified as essential components in prawn cell cycles (Braasch et al., 1999), the production of vitellogenin in protein-free media was observed (Fraser and Hall, 1999), telomerase activity was monitored (Lang et al., 2004), and comprehensive biochemical analysis of the hemolymph was performed (Shimizu et al., 2001).

Out of the 9 application studies (Alverado-Alvarez et al., 1999; Chen and Wang, 1999; Huang et al., 1999; Lynn, 1999; Lyons-Alcantera et al., 1999; Shike et al., 2000; Wang et al., 2001; Assavalapsakul et al., 2003; Maeda et al., 2004), 6 publications dealt with penaeid cells, mainly with shrimp diseases like white spot disease and yellow head viruses. These studies also were involved in antigenic characterization of hepatopancreas cells, with expression and replication of retroviral viruses in vitro, virus susceptibility, identifying the components of an inorganic physiologic buffer for cells, and the development of in vitro bioassays. In all cases the already established protocols for primary, short-term cell cultures were used to develop or to test their application aspects. This followed the attention that crustacean cell culture has gained as a potent tool for the development of diagnostic reagents and probes for use in the shrimp, crayfish, and lobster industries (Toullec, 1999).

In summary, despite numerous attempts, no established cell line of any marine crustacean has been reported to date. However, primary cultures obtained from a variety of organ sources have been reported with increasing frequency. They may represent the first steps toward the establishment of cell lines, and they provide useful information concerning the most suitable cell culture conditions for the survival and proliferative capacity of the different tissues used (Toullec, 1999).

Mollusca

In total 19 studies (6 specifically on cell culture of mollusk cells, 12 applied studies, and a single review; Fig. 1) were published from 1999 to 2004, reflecting and increase of publication numbers of more than 20% compared to the previous decade (31 publications; Rinkevich, 1999, Fig. 2).

Five out of the 6 cell culture publications (Buchanan et al., 1999; Chen and Wen, 1999; Le Marrec-Croq et al., 1999; Odintsova et al., 1999; Faucet et al., 2004) dealt with bivalves (mainly *Crassostrea* and *Mytilns*), whereas the 6 study (Sud et al., 2001) was performed on cells from the gastropod *Haliotis*. Only the study by Chen and Wen (1999) established long-term (>5 month) primary cultures of clean heart cells (also the applied study by Barik et al., 2004). Out of the 12 applied studies, 3 (Birmelin et al., 1999; Takeuchi et al., 1999; Cao et al., 2003) evaluated the expression of genes or enzymes in primary cultures of the clam *Mytilus*; 4 other publications (Domart-Coulon et al., 2000; Le Pennec and Le Pennec, 2001, 2003; Pennec et al., 2002) used primary cultures of bivalves (mainly *Crassostrea*) for studying the effects of environmental stresses and for ecotoxicologic purposes. The remaining 5 studies dealt with viral infections in *Crassostrea* cells (Boulo et al., 2000), revealing cell proliferation for the purpose of chromosome preparation in *Crassostrea* gill primary culture (Cornet, 2000), in vitro synthesis of collagen in the gastropod *Haliotis* (Poncet et al., 2000; Serpentini et al., 2000), and calcium carbonate deposition by freshwater pearl mussel's epithelial cells (Barik et al., 2004).

Although in the past mollusks were probably the most intensively studied group of marine invertebrates for the development and employment of cell cultures (Rinkevich, 1999), this intensive activity was not reflected in the recent period summarized here (1999–2004).

Echinodermata

Only 5 publications on echinodermata (4 tissue culture studies and a single review in Mothersill and Austin, 2000; Fig. 1) were issued during 1999 to 2004, which reflected a slight increase in rate of publication over the 7 publications that appeared during the previous decade (Rinkevich, 1999; Fig, 2). Two of the publications (Bulgakov et al., 2002; Odintsova et al., 2003) used a novel molecular approach in the study of invertebrate cell cultures. The authors used the transcriptional activator gene *Gal4* found in yeast to increase expression levels of the genes regulating cell growth in sea urchins and sand-dollar and thus enhance cell growth. Embryos of sea urchins were treated with plasmid DNA containing the *Gal4* gene. Expression of the transgene was confirmed by reverse transcriptase polymerase chain reaction (RT-PCR). When the fully functional gene was used, embryos effectively formed teratoma-like structures after 50 to 55 hours of cultivation. In contrast, the *Gal4* gene, devoid of acidic activating regions, demonstrated little activity as a teratogen. The *Gal4*-treated cells in blastula-derived culture showed higher DNA synthesis and higher proliferative activity than control cells. In the sand-dollar assays, cells from transfected embryos were held in vitro for up to 2 months, showing up to 4.6 times increase in numbers. The 2 other studies used sea urchin primary cultures to evaluate the development of embryonic spiculae and micromers (Hwang et al., 1999) and to elucidate the effects of lectin on adhesive and growth properties of embryonic cells (Odintsova et al., 1999).

Urochordata

Only 4 studies on urochordate cell cultures were issued during 1999 to 2004 (Fig. 1), as compared with 11 publications in the previous decade (Rinkevich, 1999; Fig. 2). These few publications dealt with different cell types of the colonial tunicate *Botryllus schlosseri* and included a single study on epithelial cell cultures (Rabinowitz and Rinkevich, 2003) and 2 applied studies on blood-cell disease (Moisseva et al., 2004) and environmental genotoxicity (Kamer and Rinkevich, 2002). A single review (Rinkevich and Rabinowitz, 2000) revealed recent trends on urochordate cell cultures. Two other studies (Mo and Rinkevich, 2001; Mo et al., 2002), although not directly dealing with tunicate cell cultures, developed the protocols for DNA extraction and 18S-rDNA identification for thraustochytrids, eukaryotic organisms that were often recorded as major contaminants in primary cultures of tunicates and other marine invertebrates (Rinkevich, 1999).

Conclusion

Reviewing the last 5 years revealed, in total, 90 references on cell cultures: 44 publications (49%) studying different aspects of in vitro culture conditions, 37 (41%) evaluating different applications of using already established methodologies, and 9 reviews (Fig. 1). This last 5-year figure represents, therefore, a 2-fold increase in the number of publications over the preceding decade (90 publications, 1988–1998; Fig. 2; Rinkevich 1999). In addition, 3 novel trends in research activities have emerged, distinguishing between the reviewed periods.

One trend is the ongoing increase of research interest in applied studies as opposed to major efforts in the past aiming to establish improved cell culture protocols (Fig. 1). Whereas in the past the vast majority of studies dealt directly with aspects of tissue culture, and only a few attempted to use existing protocols in applications, this approach has been reversed. Out of the 81 research publications (excluding review papers) during 1999 to 2004, 37 (46%) were not involved in the development of cell cultures, but in various applied aspects. This may also reflect the success of the developed protocols in attracting new research and scientific disciplines to the in vitro applicabilities of marine invertebrates. The major relevance of marine invertebrate in vitro systems to applied science is that they provide a standardized and controlled system of modeling phenomena.

Another trend is the reduced number of long-term cultures (Fig. 2). Whereas 27 (30%) of the publications in the previous reviewed decade (30%) dealt with establishment and maintenance of long-term cultures (>1 month), only 16 (18%) of recent publications revealed long-term cultures. When observing phylum-by-phylum trends, it is evident that during 1988 to 1998 (Rinkevich, 1999) long-term tissue cultures were evenly distributed between all 6 taxa studied. In contrast, long-term cell cultures during 1999 to 2004 were developed only in 3 phyla: 10 studies (36%) on crustaceans, 4 studies on sponge cell cultures, and 2 studies on mollusk cell cultures. Only 2 studies on crustacean cell cultures (Fraser

and Hall, 1999; Owens and Smith, 1999) maintained ≥10-month-old cultures.

As before, no established cell cultures from any marine invertebrate has been developed. This continued failure to establish long-lasting and proliferating cultures of cells from marine invertebrate taxa might suggest that we still lack vital information regarding marine invertebrates' cell physiology, biochemistry, and biology. It is not that the lack of progress in this discipline is related to, as was suggested (Goodwin, 1991), inappropriate comparison of marine invertebrate cell culture requirements with the culture requirements of vertebrate cells. There are probably many more reasons, and any attempt to foresee the entire range of obstacles is somewhat problematic (Rinkevich, 1999).

The third trend is involvement of cell-culture activities with fewer species of marine invertebrates. As a result a limited number of model species are being studied more extensively than in the past (*Botryllus schlosseri* for Urochordata, *Suberites domencula* for Porifera, *Penaeus* for Crustacean, *Crassostrea* and *Mytilus* for Mollusca). This phenomenon probably emanates from the success with primary cell cultures of these organisms and their importance in either basic or applied science. These contemporary research trends will probably transform the way this discipline progresses and greatly accelerate the pace of improvements in marine invertebrate cell culture techniques. They may also motivate other scientists to study the possibilities of developing proliferating cell cultures from model invertebrate species.

Approaches and protocols that have already been developed yielded successfully sterile primary cultures from many marine invertebrates. Others yielded artifacts (such as the appearance of thraustochytrids; Rinkevich, 1999). The literature of the last 2 decades (Rinkevich et al., 1994; Bayne, 1998; Rinkevich, 1999; Mothersill and Austin, 2000; this study) clearly indicates that methodologies for holding short-term, viable primary cultures have been established for a variety of marine organisms. However, numerous studies on marine invertebrate primary cultures indicate that we still need to establish protocols for the production of secondary cell cultures and cell immortalization. Similarly, as recorded in the development of insect cell cultures, patience may still be a powerful tool; many successful insect cultures have lain dormant for months before growth came about (Lynn, 1989).

Many attempts to raise cell cultures from marine invertebrates have a clear economic motive. The identification and application of novel pharmaceuticals, cosmetics, nutritional supplements, enzymes, and pigments from marine organisms have already been acknowledged. The current and potential market value of marine bioproducts is substantial. Continued discovery and development of marine resources depend on the development of techniques and protocols for cell cultures. In addition, marine invertebrate cell cultures could potentially provide in vitro systems to model the ecologic fate of xenobiotics in a variety of aquatic ecosystems. Challenging such cells with defined doses of specific xenobiotics enables the identification of biotransformed metabolites of xenobiotics near the bottom of the food chain, which could have important consequences for the health of humans and other vertebrate species (Sheehan, 2000). Adopting this approach, several studies have recently considered the use of marine invertebrate cell cultures in environmentally related studies, revealing cell toxicity, genotoxicity, and ultrastructural effects (i.e., Kamer and Rinkevich, 2002; Le Pennec and Le Pennec, 2003).

New power tools for the study of invertebrate cell cultures have recently been developed. The first are genomic and proteomic research tools that enable researchers to survey globally the alterations at messenger RNA and protein levels and to unveil their regulation. Cells under in vitro conditions may undergo alternations at genome and expression levels. It is evident that a better understanding of cellular health in vitro on the molecular level will lead to improved methodologies for culturing. Another set of power tools is mutagenesis and cell transformation in cell cultures (Crane, 1999). Recent studies have indicated that clastogenic agents such as ionizing radiation are more effective than pointing mutagens as immortalizing agents for mammalian cells. Studies with mammalian cell cultures indicated that certain mutagenic agents or prolonged treatment with a combination of mutagens might prove to be more useful in the production of immortal cell cultures (Crane, 1999). A third new tool is the use of mammalian cells as the target for developing protocols and media for invertebrate cell cultures. A good example is the Lizuka et al. (1997) study on the characterization of ascidian plasma growth factors as promoting the proliferation of mouse thymocytes.

The new trends in the discipline of invertebrate cell culture development (that include augmentation of total studies, increased use of primary cultures in applied approaches, reduced efforts in long-term cultures, and the use of fewer model species) may transform the way in which cultivation of marine invertebrates cells is used. These trends and the availability of new cellular genomic and proteomic

research tools (for example, the detection of telomerase activity in cultured cells; Lang et al., 2004) are bound to stimulate fundamental changes in views of the development of cell cultures from marine invertebrates. Insights on in vitro cellular biology gained from these novel approaches will definitely lead to a new understanding of the needs of marine invertebrate cells in vitro. A crucial requirement is the parallel development of improved media, supplementary additives, and substrates that will satisfy the needs of developing cells in vitro. I anticipate that the protocols developed and used for one taxon of marine invertebrate will be adaptable to other taxa and other invertebrate phyla. The knowledge gained from fewer model invertebrate species will have widespread implications for this entire scientific discipline, advancing as well many applied usages.

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