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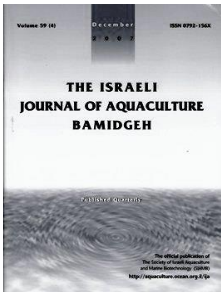

Development of Polyculture and Integrated Multi - Trophic Aquaculture (IMTA) in Israel: A Review

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Abstract

Israeli aquaculture began in the 1920s, with common carp monoculture. This was followed by polyculture of carp with tilapias, grey mullet, and planktivorous carp. Scientific research on polyculture started in the 1950s and has since contributed to the global science and practice of green water aquaculture, especially with novel polyculture approaches and concepts. Today, the industry is characterized by intensive freshwater polyculture, implemented in earthen fish ponds and reservoirs. In the Mediterranean coastal plain, fresh, brackish, and marine water polyculture is carried out in semi-intensive fishponds. Polyculture in Israel is an entrepreneurial activity that combines ecological principles of Chinese polyculture with local technologies and objectives. The Biofloc approach (active suspension ponds, ASP), periphyton, and aquaponics, were developed in the 1980s in response to rising public and policymakers' concerns and regulations on land use, pollution, use of chemicals, and organic manures. R&D on marine integrated multi-trophic aquaculture (IMTA) systems began in the early 1970s at the National Center for Mariculture (NCM) in Eilat. It started with sea bream and mullet in earthen seawater ponds, whose plankton-rich water recirculated through bivalve and macroalgae biofiltration modules. An advanced form of the concept was deployed in the early 1980s and was studied in detail using nutrient budgets. Several system models with fish, bivalves, and algae, on small and pilot scales, were studied and quantified. Abalone, sea urchins, shrimp, brine shrimp, *Salicornia,* and periphyton, were added to the Eilat marine IMTA models, beginning in the 1990s. Upon entering the third millennium, Israeli research further examined the relationship between the sustainability and economics of IMTA in world aquaculture.

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Introduction

The development of aquaculture in Israel has closely paralleled the general economic development of the country (Shapiro 2006). From the outset, Israeli aquaculture benefited from the organization and close relationship between government, business, research entities, and enthusiasts. Economic changes, privatization, ecological concerns, new methods, and new species have contributed to an expansion of the industry, from small-scale kibbutz (cooperative villages) operations producing one or two species, to large multimillion-dollar projects dealing with a multitude of species.

Israeli polyculture has been implemented in conventional earthen fish ponds and reservoirs. Typically, such water impoundments were stocked with a combination of common carp *(Cyprinus carpio),* silver carp *(Hypophthalmichthys molitrix*), tilapia hybrids (*Oreochromis aureus x O. Niloticus*), and often also grey mullet (*Mugil cephalus*). Some farms have also added grass carp (*Ctenopharyngodon idella*), red drum (*Sciaenops ocellatus*), and a hybrid silvercarp x bighead carp (*H. molitrix x H. nobilis*). Over the years, additional fresh water species were tested (Golani and Mires 2000). Entrepreneurs and research stations have cooperated closely in their research with commercial Israeli farms. Israeli scientists and practitioners have shared their expertise with other countries, particularly SE Asia (Hepher and Pruginin 1981), and South America.

Early Development of Israeli Polyculture

(adapted from Shapiro 2006)

Carp culture was introduced into Israel under the British administration during the late 1920s (Simon 2009). An experimental green water carp farm was established in 1934 on the Mediterranean coast, between Acre and Haifa (Hornell 1934). Kibbutz Nir David, near the Jordan River and south of the Sea of Galilee, began farming common carp in the late 1930s. By the end of the decade, commercial carp farming had expanded throughout the region, supported by the Jewish Agency, with the help of Yugoslavian instructors and supported by University research teams [Personal information].

In the 1940s, an outbreak of a virulent phytoflagellate in brackish water ponds threatened the infant industry. Hebrew University of Jerusalem scientists soon identified it as *Prymnesium parvum* (Haptophyta) and developed an effective treatment, using high doses of ammonium sulphate (Shilo (Shelubsky; Shilo 1953). A fish diseases laboratory headed by S. Sarig was established in 1944 at Nir David.

By the time Israel obtained national independence in 1948, aquaculture farms covered 1400 hectares and produced over 70 percent of the fresh fish consumed in the country. In 1965 Israeli fish farm production provided over half the local consumption, which peaked at 10,100 tons but decreased slightly in the following years (Sarig 1969).

Israeli polyculture began in the early 1950s with the introduction of omnivorous blue tilapia (*Oreochromis aureus*) to carp ponds (Mires 1969). Tilapia thrived in the hot climate and variable water salinity levels, and complemented the carp in its nutritional requirements (Mires 1969), thereby enhancing the ecological efficiency of the production. The Ministry of Agriculture's Department of Fisheries was involved in this research from its inception. Following initial field experiments in commercial farms (Mires 1969), the Dor Aquaculture Research Station, established on the Mediterranean coast in 1955 began investigating additional fish species - exotic species that were soon included in Israeli polyculture. The phytoplanktivorous filter-feeder silver carp was introduced in 1969 to control blooms of green microalgae, thereby improving the quality of the fish flesh. Several management practices and improved supplementary feeds (aquafeeds) were developed in the Ginnosar research station (located on the shore of the sea of Galilee), and drastically raised commercial yields from 1 ton/ha to about 6 tons/ha (Sarig 1969).

Aquaculture in the Galilee, Gilboa, and Jordan Valley regions uses both freshwater and brackish water and is characterized today by dual purpose fish ponds, integrated with crop irrigation. This approach, now a few decades old, has been a significant step in the intensification of inland fish culture in Israel. On the Mediterranean coastal plain, polyculture is carried out in fresh, brackish, and marine water fish ponds (Shapiro 2006).

In summary, Israeli polyculture is an entrepreneurial activity that, assisted by science, combines the ecological principles of Chinese polyculture with local technologies and objectives. It produces several species of fish whose complementary feeding habits maximize nutrient utilization.

Israeli Research on Polyculture

Since the middle of the 20th century, Israeli scientists such as Hepher (Hepher 1952; 1962), have contributed greatly to the global development and scientific understanding of green water and polyculture. Their work provided significant scientific information, which filled a world information gap (Coleman and Edwards 1987). [1]Yoram Avnimelech, Lev Fishelson, Balfour Hepher, Gideon Hulata, Dan Mires, Ana Milstein, Yoel Pruginin, Shmuel Sarig, Moshe Shilo, Giora Wohlfarth, and others published numerous studies on polyculture, some of which are mentioned in this review. Boaz Moav (Hinitz and Moav 1999) and Gerlad Schroeder (Schroeder 1978), also contributed significantly. Several seminal review books were published in the 1970s and 1980s (Hepher 1978; Hepher 1988; Hepher and Pruginin 1981).

Rising concerns about land use and pollution that led to stricter regulations on water disposal, and public concern about the use of chemicals and manures, raised environmental awareness in aquaculture, and led Israeli scientists to improve the sustainability of the industry (Milstein 2005), and modify the polyculture concept. In one prevalent approach, water from outdoor intensive fish ponds, raceways, and tanks was treated in sedimentation ponds and adjacent "lung" water reservoirs that recycled water back to the rearing ponds (Mires 1992; Hulata 2014). The new model consisted of several linked monoculture modules, which utilized each other's water and residues as inputs. Another development in Israeli aquaculture involved reduced intensity of the culture for better sustainability and environmental friendly purposes (Milstein 2005). Among the Israeli integrated aquaculture developments described below notably are: (a) biofloc (Avnimelech et al. 1994; Avnimelech 2006); (b) periphyton-based polyculture in freshwater (*e.g.,* Milstein et al. 2003; reviewed in Milstein 2012), and in seawater (Levy et al. 2017); (c) aquaponics (Kolkovsky et al. 2003); and (d) integrated mariculture (IMTA, see below).

(a) *Biofloc*: The development of the biofloc (active suspension ponds, ASP) approach by Avnimelech and co-workers in the early 1980s (reviewed in Crab et al. 2007) used principles taken from conventional domestic wastewater treatment. When heterotrophic bacteria and algae are grown together in well-aerated and carbohydrate (e.g., cellulose) - enriched water, they combine the inorganic fish-waste nitrogen (N) with the carbohydrate and create protein-rich flocs. These flocs are nutritious to suspension-feeders (fish and shrimp) and reduce their food conversion ratio (FCR). An efficient microbial assimilation of waste N into flocs requires a well-balanced supply of carbon (C) and nitrogen (N), and the maintenance of adequate light, oxygen, and temperature. In some biofloc studies, N recovery, by fish or shrimp doubled compared to conventional ponds (Avnimelech et al. 1994; Avnimelech 2015).

(b) *Periphyton*: The periphyton approach depends on attached aquatic organisms (mainly plants) that grow on submerged substrates installed in polyculture ponds (Milstein 2012). The periphyton food web adds significantly to the primary production by the suspended phytoplankton in the green water. Furthermore, the periphyton biomass is more concentrated and is therefore grazed more efficiently by fish and shrimp than diluted suspended food.

(c) *Aquaponics*: Israeli aquaponics has combined the culture of fish and plants (vegetables and fruit trees) in flow-through and recirculating systems (Kolkovsky et al. 2003; Kotzen and Appelbaum 2010, Appelbaum and Kotzen 2016). Commercial aquaponics with tilapia and vegetables was pioneered in the Negev Desert (Pruginin et al. 1988; Rothbard and Peretz 2002; Kolkovsky et al. 2003). Several intensive fish farms

[1] In alphabetical order

used geothermal water in fish culture raceways (tilapia and exotic fish, both ornamental and edible), and subsequently used the effluent for crop irrigation.

(d) *Integrated Mariculture Systems (IMTA)*: Early Israeli research and development of modern IMTA involved significant R&D efforts founded on traditional multi-trophic culture systems of green water aquaculture and polyculture (Hepher 1985; Kolkovsky et al. 2003). The existence of scientific and practical know-how, together with a drop in fishing in the eastern Mediterranean and a freshwater shortage in the country (particularly in the arid south), promoted the modernization of aquaculture, and the initiation of mariculture in Israel in the late 1970s (Gordin 1983; Gordin et al. 1981; Motzkin et al. 1982). These concerted activities started with the establishment of the National Center for Mariculture (NCM) in Eilat, under the umbrella of Israel Oceanographic and Limnological Research (IOLR), a government-owned research entity. From the outset, NCM focused its research on the development of sustainable mariculture, and on integrated mariculture, the combined culture of two or more marine species (Gordin 1983; Gordin et al. 1981).

Trials involved earthen fishponds, in which green (or diatom-rich brown) water recirculated through bivalves and macro algae modules. Separating the organisms into separate modules was necessary, because marine culture could involve fish, bivalves, macro-algae (seaweeds), abalone, sea urchins, and shrimp, with different nutritional requirements, life histories, and potentially conflicting culture requirements and management. The integration of several monoculture modules together with water transfer between them alleviated drawbacks of polyculture (where all species share the same water body) and allowed intensification of cultures. In an integrated farm, excrement produced in one module by a fed organism pass on to other modules and are treated by extractive photosynthetic (algae or higher) plants and filter feeder organisms (Shpigel et al. 1993a; 1993b; Chopin et al. 2001; Shpigel and Neori 2007). This approach of mariculture emerged from the concept presented in Goldman et al. (1974) and Ryther et al. (1975), on the use of domestic wastewater – seawater mixtures in marine polyculture systems with microalgae, bivalve, and seaweed modules. Although efficient and relatively inexpensive, those American efforts were discontinued, partially because of doubts as to the edibility of the products. Objections are fewer for biofilter organisms cultured in fishpond effluent (*e.g.,* Granada et al. 2015).

The interaction between fish biomass density, fish feeding, nutrient load, fish activity, water quality, light, temperature, and phytoplankton populations were first studied in earthen seawater polyculture fishponds, where the main fish was sea bream *Sparus aurata,* together with an assortment of secondary species, *i.e.,* sea bass (*Dicentrarchus labrax*), flathead grey mullet (*Mugil cephalus*), rabbit fish (*Siganus rivulatus, S. luridus*), and green tiger prawn (*Penaeus semisulcatus*). Carnivorous sea bream was fed with aquafeed, and their waste supplied the nutrients for dense phytoplankton populations. This mariculture differed from freshwater polyculture by:

(1) A continuous supply of water from the sea at a flow rate of close to half the pond volume/d; and (2) Enhancement of sulphate reduction in the sediment and thereby inhibition of methane production which is in contrast to the high methane production in the sediments of freshwater ponds.

Another study evaluated the content of organic matter, silt, and parasites in the diatom-rich water, in relation to oyster performance (Hughes-Games 1977). The water exchange rate, nutrient load, and growth rate of the phytoplankton resulted in double the chlorophyll a concentration, compared to un-stocked and unfed control ponds. Oyster trays were positioned at different locations in the ponds and in separate troughs, which collected effluent from the ponds. The oysters grew well in the subtropical seawater fish ponds (salinity 41 g/kg; temperatures up to 34^{o}C). Due to the climate conditions in Eilat, oyster growth was about 1.5 greater than that in temperate waters. The stocked oysters grew from 4-92 g in 12 months, with high product quality and survival rate.

The dynamics of plankton and nutrients in these earthen marine 'brown water' ponds and the factors that controlled water quality were determined by several studies (Motzkin et al. 1982; Krom et al. 1985a; Krom et al. 1985b; Krom et al. 1985c; Porter et al. 1986; Porter et al. 1987; Blackburn et al. 1988; Krom et al. 1989a; 1989b; Erez et al. 1990; Krom 1991). These studies evaluated the processes of planktonic and benthic photosynthesis, aerobic, and anaerobic bacterial biogeochemical processes in the sediment and in the water, together with inputs, water utilization, nutrient budgets, effluent quality, oxygen dynamics, plankton dynamics, fish metabolism, and fish health. The measured fish growth was unprecedented in marine ponds.

Clean seawater flushed nearly half of the pond volume/d and the fish (mostly seabream and mullet at a ratio of 5:1, 40,000 fish/ha) incorporated 30% of feed P and N into their flesh. The excess nutrients settled or enriched the water with dissolved nutrients, which supported phytoplankton blooms. Eventually, 70-80% of the excess nutrients were discharged in the effluent. Dissolved oxygen, temperature, and dissolved inorganic N, exhibited large diurnal cycles, which were more conspicuous in summer than in winter. Studies attributed these cycles to diurnal variation in algal activity and to the metabolism of the fish. In summer, high afternoon rates of photosynthesis led to oxygen super-saturation, high pH, and subsequent fish mortalities. Often, phytoplankton blooms 'crashed' and caused anoxia, especially before sunrise, with decreased pH and increased concentrations of ammonia. The dynamics of planktonic populations, nutrient levels, and rate of grazing by ciliates and flagellates, are related to these changes. Water quality is also influenced by bacterial metabolism in the sediment.

The suitability of seabream and shrimp (*Penaeus semisulcatus*) for growth under conditions in these ponds, as well as water quality required for adequate health and growth were defined (Kadmon 1983; Porter et al. 1986; Samocha 1986; Issar et al. 1987; Wajsbrot et al. 1989; Wajsbrot et al. 1990). These confirmed that the low-flow and intermediate-flow in earthen ponds led to progressive eutrophication (Krom et al. 1989b). Remineralisation of accumulated detritus on the bottom (Blackburn et al. 1988) led to retarded fish growth and mortality. The deterioration of water quality and the high levels of nutrients in the effluent suggest that the intensification process requires further R&D.

Integrated Mariculture (IMTA): Research and Development in the NCM campus

Original Model

In the early 1980s, the NCM moved to its permanent campus allowing interdisciplinary and elaborate R&D. The new campus is situated 600 meters north of the Gulf of Eilat (Aqaba), near the Israeli - Jordanian international border. The new NCM included several research departments, which together undertook complex multidisciplinary research, which was necessary for the development of modern sustainable marine aquaculture. The new campus included plastic-lined ponds of several designs, with hard or soft-bottoms, of different sizes. Aeration and stirring were incorporated in most of them. While most of the water overflowed from the surface, vortex stirring concentrated the detritus in the center of the ponds, from where the sludge was withdrawn into a sedimentation pond. Removal of the detritus reduced the organic load in the ponds and allowed further intensification (Neori et al. 1989; Krom and Neori 1989; Neori and Krom 1991; Gordin et al. 1990; Shpigel et al. 1993b). The original design of the new system involved a 50%/d seawater exchange and passage of the effluent into a common 250 m^3 earthen sedimentation pond. In addition, surface water from each pond was recycled through attached oyster tanks (Shpigel et al. 1993a). Each pond was stocked with 500-700 kg gilthead sea bream, which were fed high-protein aquafeed daily. The annual average growth rate was near 0.5%/d and the total annual production was 900 kg/pond (9 kg/m^{-3} /y or 90 tons/ha/y). The sedimentation pond was stocked with approximately 1000 individuals of seabream and Mozambique tilapia (*Oreochromis mossambicus*), with a total biomass of 60-100 kg, and its bottom was stocked with Manila clams (*Tapes semidecussatus*). The dissolved nutrients in the discharge from this pond to the sea were to be removed by biofiltering by a module of macroalgae ponds, but this module was never installed in full scale.

In the three growth ponds, fish assimilated 20%-30% of the feed nutrient for somatic growth. About 70% of the nutrient input was stored in the particulate phase of the water column by algal blooms. The settled detritus contained on average 17% phosphorus (P) and 10% N inputs. Intensive bacterial activity, including sulphate reduction, occurred in the detritus but not in the pond water column. Dense populations of micro-plankton developed naturally in the nutrient-rich water and dominated the particulate matter (Goldman et al. 1989; Krom and Neori 1989; Neori et al. 1989; Shpigel and Fridman 1990; Shpigel and Blaylock 1991). The phytoplankton usually consisted of a dominant alga, such as the diatoms *Nitzschia* sp. and *Lithodesmium* sp., or the green phytoflagellates *Tetraselmis* sp. and *Euglena* sp. There were also protozoa, mainly heterotrophic dinoflagellates, other flagellates, ciliates, and amoeba. A "bloom and crash" cycle of the phytoplankton community, associated with protozoan grazing of the algae, was impacted by the pond feeding regime, and occurred on a weekly or bi-weekly frequency, *i.e.,* shorter than the monthly periodicity in the earthen ponds. As the pond progressed from a bloom to a crash, the fraction of the particulate phase in the total nutrient budget dropped by about 50%. The concentrations of inorganic nutrients and chlorophyll a correlated inversely with each other, whereas pH and dissolved oxygen levels showed daily changes with magnitudes that were proportional to chlorophyll a concentration. Usually, chlorophyll a concentration in the pond ranged from medium to high (up to 0.5 g/m^{-3}), and the diurnal variation in water quality was dominated by fish excrement and phytoplankton metabolism. However, during times of algal "crash", this diurnal variation in water quality was determined predominantly by fish and heterotrophic plankton metabolism.

IMTA of Fish with Phytoplankton and Bivalves

Several multidisciplinary studies at NCM evaluated and quantified the performance of bivalves in the green and brown water integrated mariculture model (Gordin et al. 1990; Shpigel and Fridman 1990; Shpigel and Blaylock 1991; Shpigel et al. 1992; Shpigel et al. 1993a; Shpigel et al. 1993b; Shpigel et al. 1997; Neori and Shpigel 1999; Neori et al. 2001b; Shpigel 2005; Shpigel and Neori 2007). Oyster growth in tanks with water from the individual ponds was slow. Further studies revealed that oysters fed green and brown water from the sedimentation pond which received its water from several fishponds through a mutual sump (Shpigel and Blaylock 1991), grew more rapidly and condition indices were better than the oysters grown in tanks adjacent to the individual fishponds. It seems that the sedimentation pond provided better nutrition for oysters, possibly due to a more stable and diverse assortment of planktonic algae and benthic diatoms. These populations were completely different from those in the individual fishponds.

Manila clams also grew well at the bottom of the sedimentation pond. Compared with their natural habitat, the clams reared in relatively high summer temperatures (27-31^{o}C) and salinities (> 41 g/kg), thrived and grew well indicating that Manila clams are promising for IMTA. Stocking density of the oysters was kept at 25-50 kg/m^{-3} in tanks alongside the sedimentation pond. Green and brown water from the sedimentation pond was pumped at a rate of 1-2 tank volumes/h into one end of each oyster tank and discharged back as clearer water through a vertical standpipe at the other end. This design produced a continuous and laminar water flow, while allowing bio-deposits to aggregate at the bottom of the tanks. The filtration rates of the oysters and clams averaged ⅓ mg particulate organic matter (PON)/g/d. Filtration efficiency of particulate nutrient input by the bivalves was approximately 50%. Higher filtration efficiencies resulted in slower growth rates of the oysters, with a lower supply of particulate nutrients compared with the bivalve biomass. Nutrient assimilation efficiency (fraction of the filtered nutrients assimilated into bivalve biomass) was between 18%-26%. A similar fraction of the filtered particulate nutrients was regenerated as dissolved nutrients, and additional 16%-22% were converted into bio-deposits. Oysters and clams reached commercial sizes of 80-120 g and 10-16 g in 16 and 18 months, respectively, with average daily growth rates of 0.5% and 0.6%, respectively. Both species exhibited relatively high condition indices (dry meat/dry shell x 100) throughout the year. Annual mortality averaged 20%. Bivalves produced in the integrated mariculture system were free of human pathogens and fit for human consumption.

The ecological function of the bivalves in the system was similar to that of silver carp in freshwater polyculture, namely the uptake from the water of suspended particulate organic matter, mostly phytoplankton. Clarifying of pond water of particulate matter by the bivalves allows a significant decrease in water exchange and, proportionally a reduced discharge of nutrients to the sea. Water clarification by the bivalves also renders the water adequate for macroalgae culture. The overall yield of the three crops in relation to feed input can be two to three times higher than the yield of a modern net pen fish farm. The commercial value generated by the bivalves and the macroalgae may be significant, since at least 60% nutrient input in the fish farm could be assimilated by such crops (Shpigel et al 1993b). Anticipated average annual yields of the system (recalculated for a hypothetical 1 ha farm) are 25-35 tons of seabream, 50-100 tons of bivalves, and 30-125 tons of fresh macroalgae. Such farms require highly trained and experienced staff to balance the biological processes involved, since one must be aware of the risk stemming from the fact that all components of this system are linked and therefore depend on one another.

Fish and Macroalgae: a Simple Model

Macroalgae-based integrated farms alleviate limitations involved with green water systems. While plankton populations cloud the water, may bloom and crash, and can be washed out by excessive water exchange, macroalgae keep the water relatively transparent. Macroalgae can be easily harvested and their density maintained quite easily and independently of the water exchange rate. Experimental and theoretical studies that led to the introduction of macroalgae into the NCMs IMTA concept originated in Israel in the mid-1980s and continue to this day (Friedlander and Zelikovitch 1984; Vandermeulen and Gordin 1990; Cohen and Neori 1991; Neori et al. 1991; Gonen et al. 1994; Friedlander and Levi 1995; Israel et al. 1995; Ellner et al. 1996; Dvir et al. 1999; Neori 1991; Neori 1996; Neori 2008; Krom et al. 2001; Schuenhoff et al. 2003; Msuya and Neori 2008; Figueroa et al. 2009; Figueroa et al. 2010; Ben-Ari et al. 2014; Samocha et al. 2015; Shpigel et al. 2016).

The growth of several macroalgae species was evaluated as a function of essential environmental conditions, such as light intensity, temperature, water motion, plant density, and nutrient (N, P) enrichment with fertilizers and fishpond effluent. Species of the cosmopolitan genera *Ulva* and *Gracilaria* proved to be most suitable for these cultures. Culture of macroalgae in fishpond effluent began in the late 1980s. The approach of intensive suspension (also known as 'tumble') cultivation of macroalgae in tanks and ponds (Hanisak and Ryther 1984) was selected because it provided high yields and was easily controlled and mechanized. A vegetative clone of *Ulva lactuca* was stocked in bottom-aerated tanks, which were flushed with fishpond effluent. Stocking densities and nutrient supply were optimized for long-term high yields, which matched the best ever reported for any plant, 30-55 g dry weight/m^2/d. Sedimentation pond water and effluent from bivalve tanks also supported good macroalgae growth. The growth rate of the macroalgae was linked to stocking density, water exchange rates, water nutrient content, and intensity of aeration. With proper adjustment of nutrient supply to macroalgae practically all the ammonia-N and a large fraction of P in the effluent were assimilated into harvestable *Ulva* biomass. With ammonia N fluxes (inflow rates) of 8 g/m^2/d, assimilation efficiency ranged around 50%. Night-time ammonia removal by the algae was proportional to their level of N-starvation. The macroalgae produced were rich in protein, up to 45% in dry weight. Seabream fingerlings and oysters seemed to prefer macroalgal tank effluent over clean Red Sea water. Since 2000, new studies have developed a promising three-stage diminishing-size macroalgae biofilter system for the culture of *Ulva* in fishpond effluent. This system has provided a high yield of protein-rich *Ulva*, together with an efficient nutrient removal from the water (Neori et al. 2003; Schuenhoff et al. 2003; Msuya and Neori 2010).

The success in the culture of macroalgae in effluent from marine fishponds prepared the ground for the development of a simple fish-macroalgae IMTA model, where relatively clear fishpond water was recirculated through macroalgae ponds (Neori 1991; Neori et al. 1993; Neori et al. 1996; Krom et al. 1995; Chopin et al. 2001; reviewed in Neori et al. 2004). The macroalgae simultaneously removed most of the ammonia excreted by the fish, converted it into a commercially valuable easily harvestable protein-rich biomass, and stabilized water quality (oxygen, pH, turbidity and ammonia). The effluent passed a polishing stage in the form of a smaller macroalgae tank, which removed the remaining ammonia and also nitrate and nitrite. A model consisting of several tanks and a pilot consisting of 100-m^3 (100-m^2) ponds were studied for several years. Both systems maintained stable and fish-safe water quality, and produced effluent quality that was superior to that of the green and brown water IMTA systems described above. The design allowed significant increases in overall water residence time (up to 5 days), i.e., a reduced exchange rate with the sea, and produced a high yield of high-protein macroalgae in addition to the fish. Farmed *Ulva* in its fresh, dried, and frozen forms has been gaining markets as a nutritious and sustainable human food, and as an enrichment ingredient of ornamental fish aquafeeds (*e.g.,* Mazarrasa et al 2014). A seabream – *Ulva* farm of this model is expected to produce at a minimum 55 tons of fish and 385 tons fresh weight of macroalgae/ha/y, together with clean effluent. Calculations for a salmon and *Gracilaria* farm in Chile predict the production of 92 tons of fish and 500 tons fresh weight of *Gracilaria*/ha/y.

Fish with Macroalgae and Macroalgivores (Abalone and Sea Urchins)

The availability of fresh protein-rich macroalgae from IMTA led to the evaluation of a macroalgivore as a secondary animal crop in the fish-macroalgae IMTA farm (Kissil et al. 1992; Shpigel and Neori 1996; Shpigel et al. 1996a; Shpigel et al. 1996b; Shpigel et al. 1999; Neori et al. 1998; Neori et al. 2000; Neori et al. 2001a; Neori et al. 2001b; Lee et al. 2004; Nobre et al. 2010). The basic design consists of interconnected modules for the culture of fish, macroalgae, and macroalgivores (abalone and sea urchins). The macroalgivore *Siganus* sp. is a further option (*e.g.,* Ben-Tuvia et al. 1973; Xu et al 2011), to be examined in the future. These modules can be arranged in different ways, according to location, market, operational, and other requirements or constraints. NCM's research provided designers with optimal dimensions, stocking densities, operation protocols, expected yields, and proximate revenue structures for several such farm models. One simple design uses macroalgae ponds that are fertilized with inorganic nutrients to feed macroalgivores in nearby tanks; the nutrient-rich effluent passes through or recirculates through the macroalgae module, where the nutrients are recovered. Thus, the macroalgivore module provides the macroalgae module with water and some nutrients, while the macroalgae module provides the macroalgivore module with both food and biofiltration. This model has been implemented with economic success in South Africa, where kelp (*Eklonia*) is fed, together with farm-grown *Ulva,* to the abalone, and fertilizers serve as an exogenous source of nutrients (Nobre et al. 2010). This IMTA design is simple, operationally compact, with low construction and operational costs. It was more profitable than a control abalone monoculture operation on the same farm.

A three-species system incorporates the culture of fed finfish, such as seabream, into the simpler macroalgae-abalone farm. In this design, as it was implemented at the Seaor Marine farm (below), seawater flowed through abalone modules and consequently drained through the aquafeed-fed fishponds into macroalgae ponds, and then discharged or recycled into the fishponds. Aquafeed supplied most of the nutrients to the Seaor farm. The different modules were scaled so that fish excrement, macroalgae nutrient uptake, production of protein-rich macroalgae biomass, and abalone macroalgae nutrition requirements were all well-proportioned. The farm initially produced fish and abalone that used the entire macroalgae production with little waste (Neori and Shpigel 2006; Nobre et al. 2010).

Fishponds with Biofilters of Constructed Wetlands with Salicornia Spp.
Several species of the halophyte *Salicornia* are valuable fresh vegetable crops that thrive in harsh saline conditions, including seawater (Ventura and Sagi 2013). *Salicornia* (glasswort, pickle weed, marsh samphire) has been incorporated into one system with intensive fishponds (Bunting and Shpigel 2009; Ventura et al. 2011: Shpigel et al. 2013). IMTA system with a constructed wetland, such as the *Salicornia* wetland developed at NCM, suggests the use of soil or gravel as a substrate (Shpigel et al. 2013; Ventura et al. 2015). The valuable by-product, *Salicornia* shoots, has been established in growing markets of health food, nutraceutical, and beauty industries (e.g., Hortidaily.com 2015). Water treatment efficiencies and *Salicornia* production were studied and analysed, technical limitations have been defined, and conditions required for high biofiltration efficiency were identified (Shpigel et al 2013). The constructed wetlands are adaptable to marine operations with limited water flow and sufficient nutrient concentrations, i.e. hatcheries and land based recirculated marine fish farms.

Fish and Periphyton, a Low-Cost Model
Fish and shrimp culture with periphyton perhaps presents the cheapest model of integrated aquaculture. Periphyton is a plant-dominated community that develops on pre-fixed substrates, when they are introduced to the upper water column of a fertilized polyculture pond. Periphyton biomass is a nutritious addition to the planktonic biomass of normal polyculture ponds for feeding various fish such as carp (Azim et al. 2001 and 2002), tilapia (Milstein et al. 2005), catfish (Amisah et al. 2008) and freshwater prawn (Asaduzzaman et al. 2009). In experiments with tilapia, periphyton reduced commercial feed requirements by 40% with no deterioration in either fish yield or water quality (Milstein et al. 2008; 2009). Promising results in penaeid shrimp growth, survival, and production were also observed using periphyton in brackish water (Anand et al. 2013; Banerjee et al. 2010; Khatoon et al. 2009). Periphyton grazing by mullet, sea bream, and other fish has been observed in their natural habitat (Ferrari and Chieregato 1981) and in mariculture (Batzina et al. 2012). A recent development at NCM includes the use of periphyton as biofilter for effluent of semi-intensive mullet culture ponds (Levy et al. 2017), and as food for mullet fingerlings (unpublished).

In contrast to macroalgae biofilters, where ammonia-nitrogen uptake is favoured and where ammonia inhibits nitrate removal (Neori 1996), periphyton has also been suggested to support the simultaneous removal of ammonia and nitrate. The latter is assimilated or denitrified to N_2 gas (Axler and Reuter 1996). Research on an integrated biofilter that involves *Ulva* sp. for ammonia removal and a post treatment unit of periphyton for nitrate removal has been established recently at NCM and has shown an effective uptake of total N and total P from effluent (unpublished).

Integration of Detritivorous Fish with Seabream Cage Culture in the Red Sea
The culture of omnivorous flathead grey mullet below fish sea cages recycled organic waste that settled and accumulated on the bottom in a Red Sea cage farm (Porter et al. 1996; Katz et al. 2002; Lupatsch et al. 2003). The mullet were contained in benthic enclosures open to the underlying sediment and fed only uneaten feed and undigested feces. The fish effectively removed organic C, N, and P from the organically enriched sediment and grew at a rate equivalent to that of mullet reared in brackish inland water ponds. Eventual scaling up of such systems to a pilot or commercial scale operation would involve the deployment of large bottom enclosures for the mullet.

A Complex Multi-Species Model
A multi-species design can combine the integrated farming of organisms from several fish or shrimps, fed in green or brown water with plankton, filter feeders, macroalgae, macroalgivores and detritivores (Fig. 1). This design provides a selection of optional modules for each trophic level, depending on the choice of the farmers. A reduced number of trophic levels, as described earlier, can also be selected from this “basic” design. The selection of modules depends on factors such as economics, the size of the

farm and the expertise that it can provide, as the increased flexibility requires highly skilled manpower and sophisticated management.

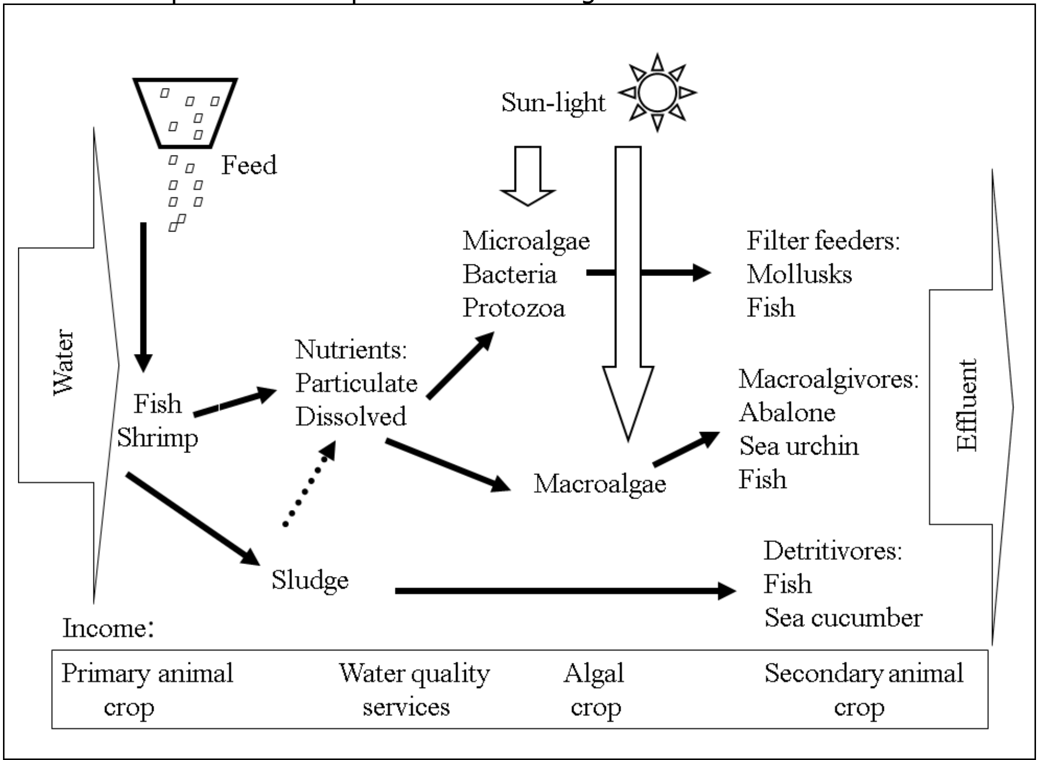

Fig. 1. A diagram of optional nutrient pathways to crops and waste in the concept of IMTA, as developed at NCM**.**

A multi-species design can combine the integrated farming of organisms from several trophic levels - fed fish or shrimp, greenwater / brownwater plankton, filter feeders, macroalgae, macroalgivores and detritivores (Fig. 1). This design provides the grower with a selection of modules for each trophic level, depending on the choice of the growers. A reduced number of trophic levels, as described earlier, can also be selected from this "mother" design. The selection of modules would depend on the size of the farm, the level of expertise available, as long as the farm is ecologically-balanced and profitable. The economic benefits of the complexity hinge on product diversity and farm resilience. Nutrients are supplied by fish (or shrimp) aquafeed, and biofiltration is performed by other organisms. Greenwater / brownwater effluent and benthic diatoms both feed filter-feeder clams and oysters (as well as tilapia, milkfish, etc.). The macroalgae module feeds the macroalgivores. Detritivores feed on feces and other settled organic matter.

Diversity and resilience comes at the cost of requiring larger pond areas than the three-species model for the same total production of animal products. Furthermore, this design depends on microbial processes that occur in the different ponds for its successful operation. These processes can be location-dependent, and should therefore be characterized and sufficiently understood for a successful operation. Finally, increased flexibility is achieved by increased complexity, which requires highly skilled manpower and sophisticated management.

Experimental examination of the multi-species model by the NCM was expanded in stages, until it was implemented as three, four, and even five species models in the commercial farm, SeaOr Marine, 40 km north of Tel Aviv (see below). The fish-plankton-bivalve model was implemented semi-commercially in another farm, PGP 1994 Ltd., in Eilat.

Socio-Economics, Governance and Regulations

A recent European effort that involves Israeli scientists has examined socio-economics, governance, and regulations issues that have a bearing on the European development of IMTA (Alexander et al. 2015; Alexander et al. 2016). Research has identified the variable levels of awareness of IMTA as an inhibition to its development in Europe. It was further proposed that waste utilization and economic benefits by IMTA farms in comparison with monoculture of fish and other organisms were helping their development, so that their overall image was positive and could promote the aquaculture industry. However, unfounded legislation and regulation of such farms, the requirement of IMTA farms for large areas, their perceived and often unfounded involvement in disease outbreaks, and food safety issues, were a hindrance. A dialogue with the different stakeholder groups and countries was suggested as the proper course to promote the development of IMTA in Europe.

Commercial Enterprises

SeaOr Marine Enterprises Ltd.

The company operated for several years on the Israeli Mediterranean coast north of Tel Aviv, where it cultured marine fish (gilthead seabream), macroalgae (*Ulva* and *Gracilaria*), and Japanese abalone (*Haliotis discus hannai*). It leveraged local climate and recycled fish waste products into macroalgae biomass, which was fed to the abalone. It also effectively purified the water sufficiently to enable it to be recycled to the fishponds and meet point-source effluent environmental regulations. The farm was a pilot that operated well, technically, but was too small to make a profit. New owners (Sakura Products from Nature and later Seakura - http://www.seakura.net/) have focussed on macroalgae farming, particularly *Ulva* and *Gracilaria,* for fresh and processed human food.

PGP 1994 Ltd.

The fish-plankton-bivalve model was implemented semi-commercially for several years in in Eilat. It produced microalgae-bivalve/brine shrimp that grew in effluent from marine fishponds. Surplus nutrients from fish culture supported dense microalgal populations, which fed bivalves (*Crassostrea gigas* and *Tapes semidecussatus*), that grow on the bottom of sedimentation ponds or in rearing tanks. Brine shrimp (*Artemia* sp.) were also produced at high rates on these microalgae. The bivalves reached commercial size in 18 months, with average daily growth rates of 0.8%. Annual mortality averaged 20-30%. Final stocking density was 8-10 kg/m^2. Brine-shrimp yields reached from 1.8-3.6 kg/m^2/y (Neori et al. 2001b and unpublished results).

Conclusions

Early research and development on green-water aquaculture and on polyculture in Israel and the establishment of the NCM in Eilat set the stage in human and institutional resources for rapid and significant advances. The change in emphasis from freshwater single-pond microalgae-fish systems, as in Asian polyculture, to compartmentalized (modular) marine systems of fish, microalgae, bivalves, macro-algae, and macroalgivores, was the result of a logical scientific progression.

This research demonstrated how algal and bivalve biofilters could control the major water quality problems in intensive culture of fish and shrimp, while improving effluent quality and recycling most of the waste into valuable crops of animals and algae. The IMTA concept developed in Israel is modular (Fig. 1) and flexible in the allocation of nutrient resource shares to a variety of products, based on operational and economic considerations. Seawater is pumped from the sea to ponds containing fish or shrimp fed with a pelleted diet. The effluent water from these ponds, rich in organic matter and inorganic nutrients, can be stripped of nutrients in green and brown-water ponds containing microalgae, or macro-algae. Each additional product adds to the resilience of the IMTA farm to internal (disease) and external (market price) fluctuations (Neori and Shpigel 2006; Nobre et al. 2010).

Commercial IMTA farms on a full scale have not developed in Israel, probably due to bureaucracy, ignorance, and entrepreneurial failure. However, the mixed culture of fish, molluscs, and macro-algae, practiced in the coastal bays of China, the culture of abalone and macro-algae in South Africa, and the culture of fish, mussels, and kelp in Canada are growing industries that provide good examples for the technical validity and economic viability of IMTA.

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