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## Biological Science

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Coral Reef Ecosystems

Report of Family Physidae

Highlights

Identification of Pathogenic

Mathematics for Biological Sciences

Discovering Thoughts, Inventing Future

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## Marine Plants in Coral Reef Ecosystems of Southeast Asia

By E. A. Titlyanov, T. V. Titlyanova & M. Tokeshi

*Zhirmunsky Institute of Marine Biology*

*Corel Reef Ecosystems*- The coral reef ecosystem is a collection of diverse species that interact with each other and with the physical environment. The latitudinal distribution of coral reef ecosystems in the oceans (geographical distribution) is determined by the seawater temperature, which influences the reproduction and growth of hermatypic corals – the main component of the ecosystem. As so, coral reefs only occupy the tropical and subtropical zones. The vertical distribution (into depth) is limited by light. Sun light is the main energy source for this ecosystem, which is produced through photosynthesis of symbiotic microalgae – zooxanthellae living in corals, macroalgae, seagrasses and phytoplankton.

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# Marine Plants in Coral Reef Ecosystems of Southeast Asia

E. A. Titlyanov <sup>α</sup>, T. V. Titlyanova <sup>σ</sup> & M. Tokeshi <sup>ρ</sup>

## I. CORAL REEF ECOSYSTEMS

The coral reef ecosystem is a collection of diverse species that interact with each other and with the physical environment. The latitudinal distribution of coral reef ecosystems in the oceans (geographical distribution) is determined by the seawater temperature, which influences the reproduction and growth of hermatypic corals – the main component of the ecosystem. As so, coral reefs only occupy the tropical and subtropical zones. The vertical distribution (into depth) is limited by light. Sun light is the main energy source for this ecosystem, which is produced through photosynthesis of symbiotic microalgae – zooxanthellae living in corals, macroalgae, seagrasses and phytoplankton. Therefore, hermatypic corals are able to win in competitive struggle for the substrata and resources with other autotrophic organisms even under light about 1% of the surface photosynthetically active radiation (PAR<sub>0</sub>). The hard substratum for the majority of recent coral reefs is limestone basis, which is formed from remnants of historical reefs (fossil) appeared on the earth in the middle Triassic period (225–200 million years ago). The other hard substrata colonized by hermatypic corals are underwater rocks and stones, which will further form coral reefs. Moreover, coral reef can also be built on the basis of artificial substrata (e.g. oil towers or underwater constructions of mariculture farms in tropical regions of the oceans) (Titlyanov, Titlyanova, 2012a). The main difference between coral reefs and other underwater ecosystems is the formation of hard substratum, which is based on hermatypic coral colonies dying off and subsequent colonization by animals including corals and seaweeds. Coral reefs are the most diverse ecosystem and provide the largest primary production among all the underwater ecosystems on the coastal shelf. They occupy less than 0.1% of the world's ocean surface, but provide habitat and refuge for 25% of all marine organisms, including fish, mollusks, worms, crustaceans, echinoderms, sponges, tunicates, etc. Competition for resources such as food, space and sunlight are the primary determining

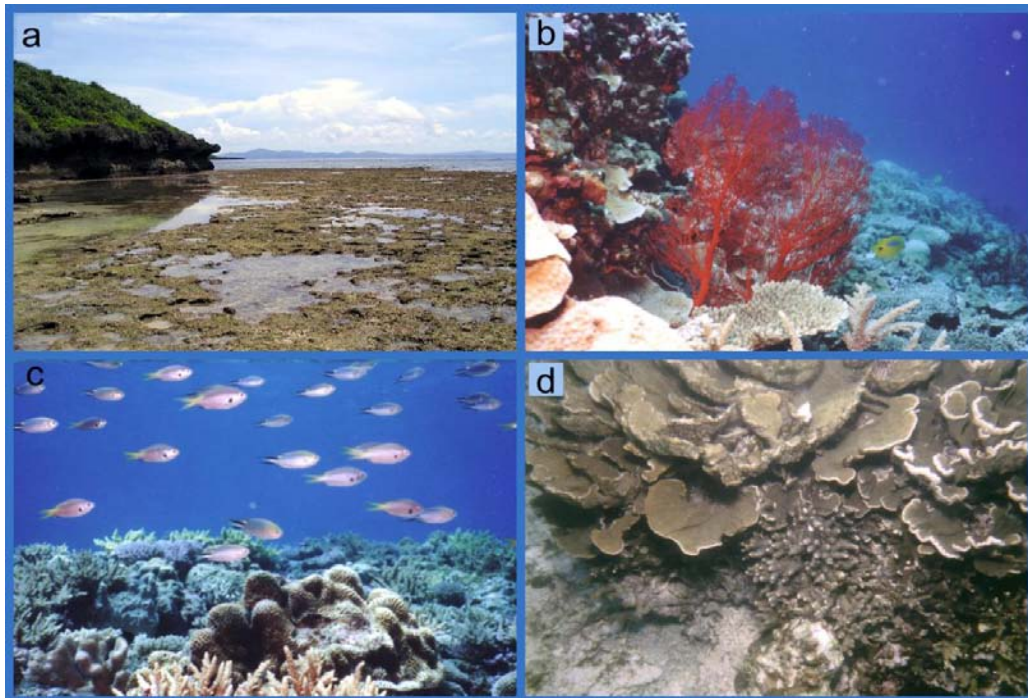
factors for the organisms' abundance and diversity on a reef.

Coral reefs have calcium carbonate based structures that are constructed by communities of reef building stony corals or scleractinian corals. Coral reefs are generally divided into four main types (Littler, Littler, 2003): (1) fringing reef is the most common type that develops adjacent and parallel to the shoreline; (2) barrier reef is an actively growing type that also occurs parallel to the coastline but relatively further away from the shore; (3) atoll is a ring of calcareous reefs that is often interspersed with low sandy isles and a relatively shallow, sheltered lagoon; (4) patch reef occurs as small mounds or cup-shaped structures growing on hard substrates that cast into the lagoons of barrier reefs or atolls (Figs 1–4).

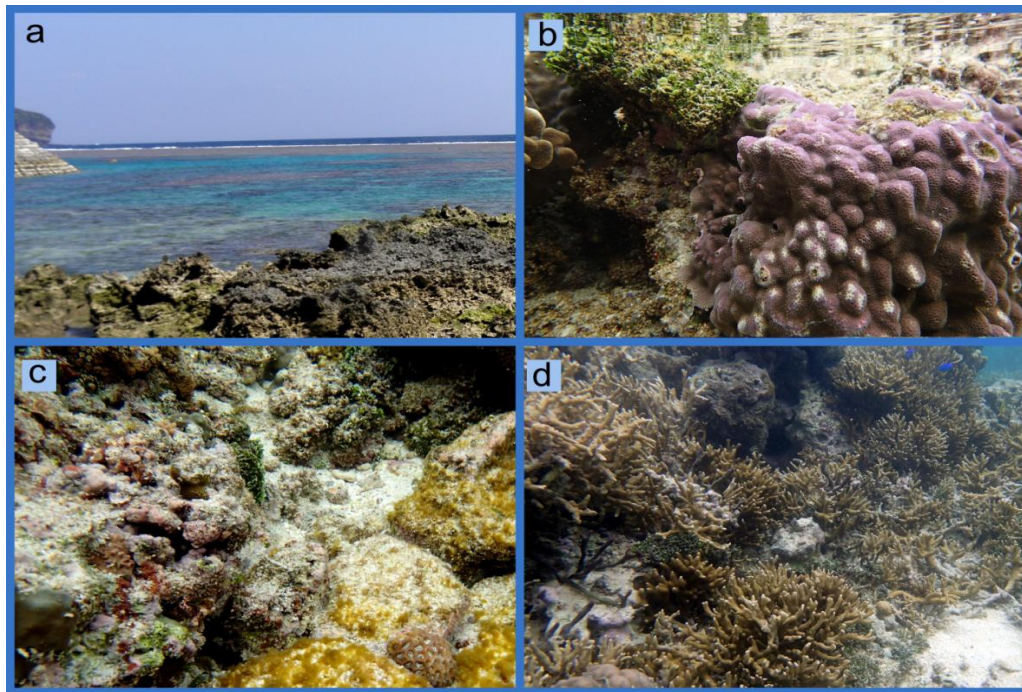
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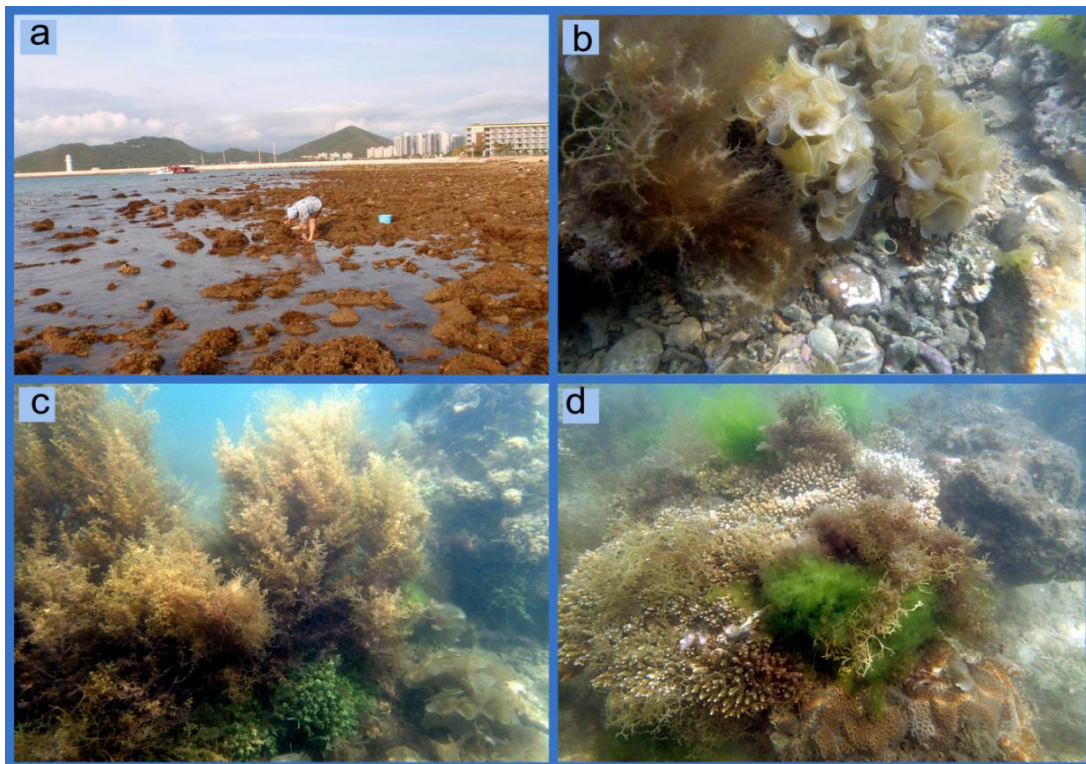




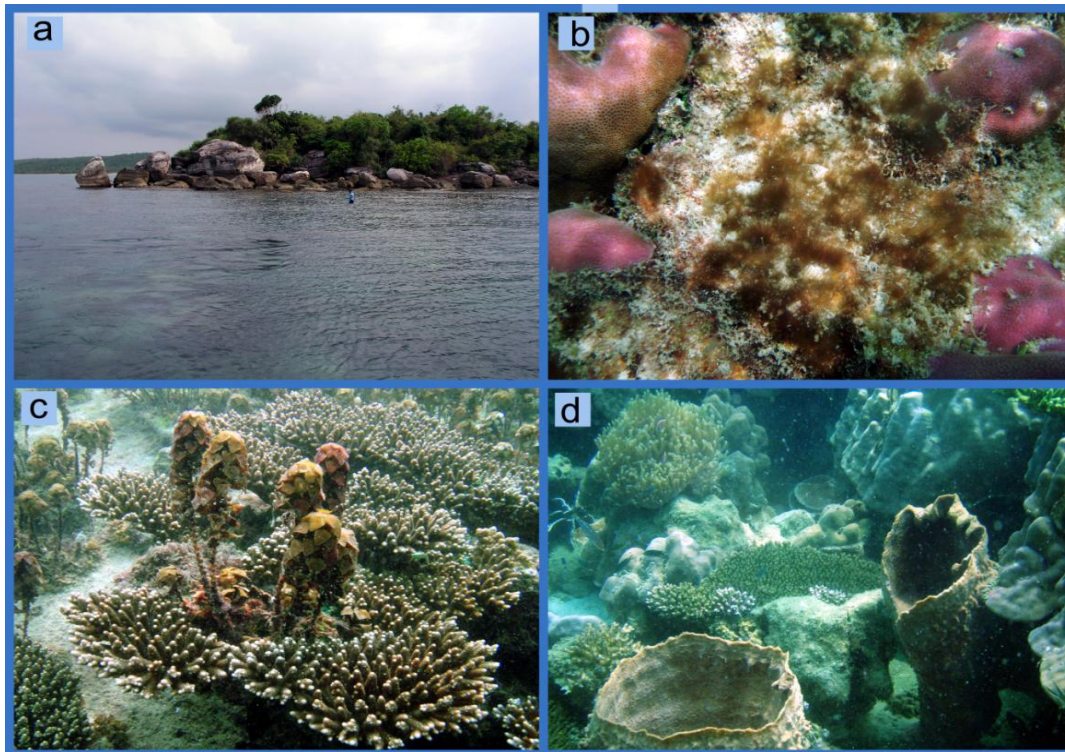
*Fig. 1:* Fringing reef located along the East coast of the island Sesoko (Okinawa Prefecture, Japan). Sesoko Island ( $26^{\circ}39'N$ ,  $127^{\circ}51'E$ ) is situated not far from the coast of Motobu Peninsula of Okinawa Island and it should seem similar to large number of islands of the Ryukyu Archipelago, June 1995: a – coral reef at low tide; b – coral and calcareous algal communities on the reef slope; c – coral and algal communities on reef flat; d – coral community in lagoon.



*Fig. 2:* Fringing reef located along the southern coast of Yonaguni Island ( $24^{\circ}27'N$ ,  $122^{\circ}57'E$ ), which is situated at the tropical northern periphery of the Indo-Pacific Ocean. The island is one of the Yaeyama Islands and the last southwest island in the Ryukyu Archipelago chain (Okinawa Prefecture, Japan), March 2013: a – coral reef in Higawa Bay (in the foreground fossil reef; in the background of live coral reef at high tide); b – coral and algal communities on reef crest; c – coral and algal communities on reef flat, d – community of branched hermatypic corals in lagoon.



*Fig. 3:* Heavily damaged coral reef located along the southern coast of Hainan Island, China (Luhuitou Peninsula, 18°13'N, 109°34'E), March 2012: a – fringing coral reef at low tide; b – algal community in the middle intertidal zone; c – algal community in the low intertidal zone; d – coral-algal community in the upper subtidal zone.



*Fig. 4:* Coral reef along the coast of Thom Island (9°59'N, 104°01'E), one of group of the An Thoi Islands and the Phu Quoc Archipelago (Vietnam) in the Gulf of Thailand, March 2009: a – reef at high tide; b – coral-algal community in the intertidal zone; c, d – communities in the upper subtidal zone.

The first encountered zone of reefs is on the beach or rocky intertidal. It occurs adjacent and parallel to the intertidal shoreline, consisting of a shallow reef platform followed by a lagoon. The depth of the lagoon varies from less than a meter to 10–30 meters. The lagoon with a sediment bottom is protected from intense wave actions by the offshore barrier reef. Channels that connect the lagoons with the open waters provide fresh and cold water for the lagoons. The channels enter lagoons via reef flat, a broad, shallow and flat part of a reef, which is protected from heavy surf by reef crest. Reef crest is the top of the reef slope, which descends as deep as 5–30 meters (Littler, Littler, 2003).

Coral reefs are less resistant to natural and anthropogenic catastrophes, which disturb Previously established balance for primary production between producers and consumers, predators and tolls, and symbionts and their hosts. This notion indicates that fluctuations in the abundance of one species can drastically alter the diversity and abundances of the others. Hurricanes and other large storm events can be the stimulus for such alterations, but anthropological forces are actually more common for influencing the ecosystem. For example, overfishing of herbivorous fish often results in increased growth of algae and seagrasses. The disturbance of symbiotic interactions between corals and algae-zooxanthellae leads to coral bleaching and their subsequent mortality. Dead coral colonies are rapidly colonized with algae, which causes the biodiversity of coral reef shift to plant reef with the predominance of macrophytes in the ecosystem. The damaged coral reef may completely recover, or recover to a different state, which is dominated by other species and forms of corals, or turn into “plant” reef. After decades, this “plant” reef can be destroyed by waves due to the mortality of hermatypic corals and the loss of the main and unique property to build carbonate calcium for reef formation.

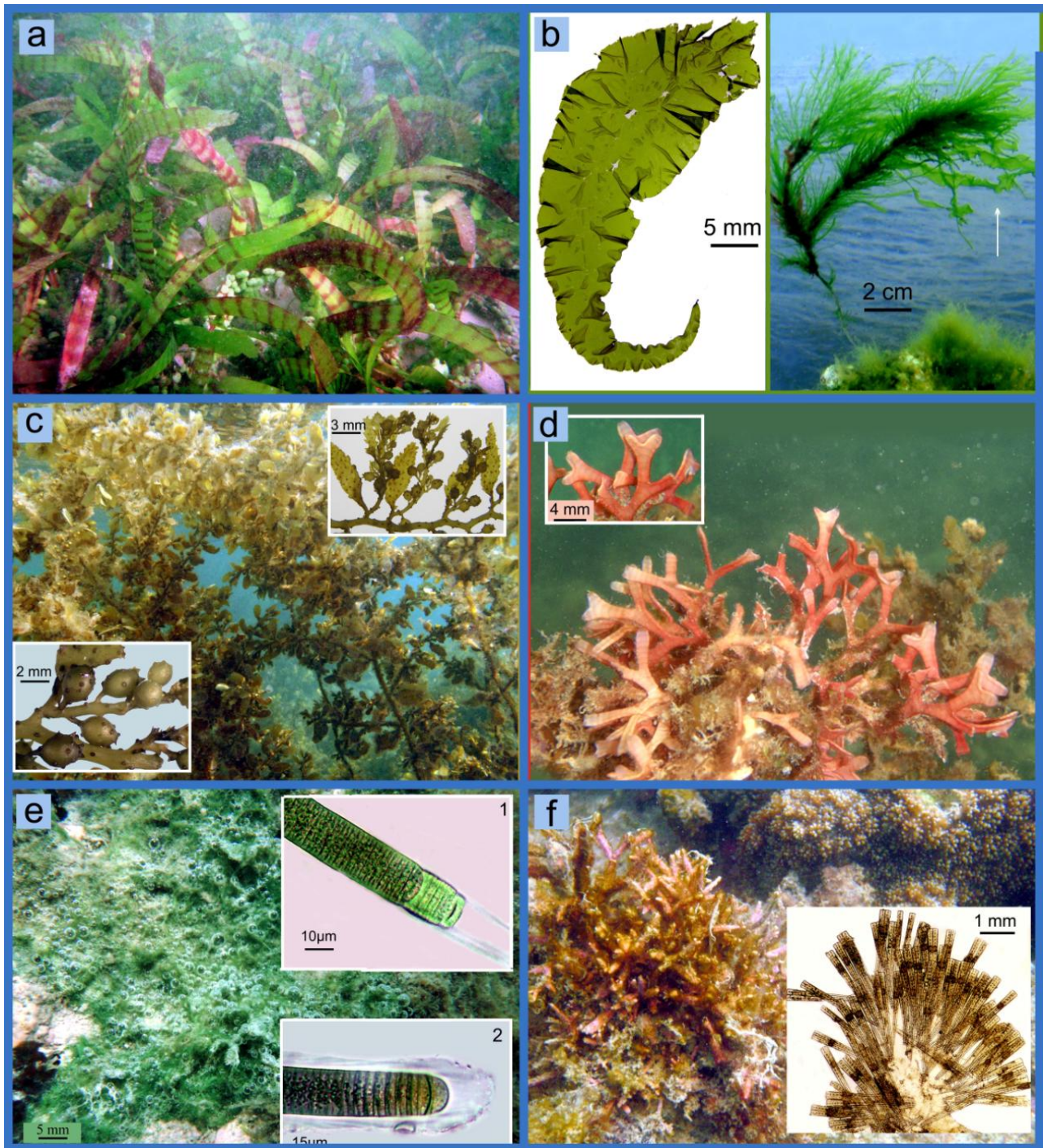
## II. MARINE PLANTS OF CORAL REEFS

All the marine plants are autotrophic organisms (in independence of their systematic status). They contain chlorophyll a and execute photosynthetic process, which produces organic matter and oxygen by absorbing sunlight, carbon dioxide and water. Marine plants belong to three groups of organisms: microalgae (blue-green, diatoms, dinoflagellates, zooxanthellae), macroalgae (green, brown and red) and higher plants or seagrasses. The latter two groups are

- a) *Identifying the characteristics of representatives from different Phyla*
  - i. *Phylum Tracheophyta, order Alismatales (sea-grasses)*

Seagrasses (marine flowering plants), inhabiting sandy and silty-sandy areas of the bottom, are one of the main components of reef ecosystem. Some of them

are able to attach to the hard base of the reef (e.g. *Thalassodendron ciliatum*). Seagrasses can form extensive beds or meadows in shallow sheltered coastal waters. Similar to the terrestrial higher plants, seagrasses have roots, stems, leaves and flowers (Fig. 5a).



**Fig. 5:** Identifying the characteristics of representatives from different phyla: a – the seagrass *Cymodocea serrulata* (R. Brown) Ascherson and Magnus (Phylum Tracheophyta, Order Alismatales), Phu Quoc Island (Vietnam) in the Gulf of Thailand, April 2009; b – *Ulva linza* Linnaeus (Phylum Chlorophyta, Order Ulvales), plants growing on the nylon cord, Hainan Island, Luhuitou, April 2012; c – *Sargassum polycystum* C. Agardh (Phylum Ochrophyta, Order Fucales), in habitat, the upper subtidal zone, Hainan Island, Luhuitou, April 2012. Insets: details showing air bladders and phylloids; d – *Dichotomaria marginata* (J. Ellis and Solander) Lamarck (Phylum Rhodophyta, Order Nemaliales), in habitat, the upper subtidal zone, Hainan Island, Luhuitou, April 2012; e – community of Cyanobacteria in the low intertidal zone (Sanya Bay, Hainan Island). Insets: 1 – *Planktothrix agardhii* (Gomont) Anagnostidis and Komárek; 2 – *Lyngbya majuscula* Harvey ex Gomont (Phylum Cyanobacteria, Order Oscillatoriales), the upper subtidal zone, Hainan Island, Luhuitou, April 2012; f – the diatom alga *Licmophora* sp. (Phylum Bacillariophyta, Order Licmophorales) – epiphytic on the red alga *Amphiroa foliacea*. Sanya Bay, March 2012.

ii. *Phylum Chlorophyta (green algae)*

The green algae (Fig. 5b) are named for their green chloroplasts. They are characterized by the predominance of the green pigments (chlorophylls *a* and *b*), which mask carotenes, xanthophylls (such as lutein, zeaxanthin and siphonoxanthin) and other pigments. These photosynthetic pigments are located in chloroplast's thylakoids that are grouped into stacks. The green algae's cell wall is composed of a layer of pectin and an inner cellulose layer. Starch is their storage nutrient. Most green algae reproduce both sexually and asexually. The reproduction involves the formation of flagellated spores and the production of non-flagellated spores to a less degree. Alternation of generations, where the algae alternate between gametophyte and sporophyte, is common in the multicellular green algae. Vegetative reproduction through the fragmentation of thalli is also common, especially in filamentous forms (Dawes, 1998).

iii. *Phylum Ochrophyta, Class Phaeophyceae (brown algae)*

The brown algae (Fig. 5c) contain large amounts of carotenoids in their plastids, which are brown and golden pigments that give the plants their characteristic color. The most important carotenoid in the phaeophytes is fucoxanthin. Besides, their plastids also contain other pigments such as chlorophylls *a* and *c*. The chloroplasts have thylakoids and 3 of which are grouped into a stack. Their cell walls are mainly composed of cellulose and alginic acid. Laminarin and mannitol are the main forms of nutrient storage of phaeophytes. Laminarin is a polymer of glucose and mannitol is a six-carbon sugar alcohol. The life cycles of brown algae vary greatly. They have both sexual and asexual reproduction. Vegetative propagation can also happen through the fragmentation of thalli. Additionally, there is a formation of special reproductive branches known as propagula present in species of *Sphacelaria* (Dawes, 1998).

iv. *Phylum Rhodophyta (red algae)*

The red algae (Fig. 5d) are characterized by dominant pigments phycoerythrin and phycocyanin, which give this group their red coloration and mask the color of the chlorophylls *a* and *c*. Other pigments found in their cells are carotenes and xanthophylls (lutein, zeaxanthin, etc.). Chloroplasts of the red algae have thylakoids that occur individually (not in stacks as in the green and brown algae). Their cell wall contains less cellulose and more of gelatinous or amorphous sulfated galactan polymers, such as agar, carrageenan, etc. Their storage nutrient is floridean starch. The red algae are reproduced in various ways. Their reproduction methods and life histories are the most complicated which include both sexual and asexual modes. The majority of advanced genera have one gamete-producing phase (sexual phase) and two spore-

producing phases (asexual phase). Vegetative multiplication also occurs in the Phyla (Dawes, 1998; Abbott, 1999; Lee, 2008).

v. *Phylum Cyanobacteria (the blue-green algae)*

Blue-green algae, known as Cyanobacteria, are named for the blue-green pigment phycocyanin, which gives them the blue-green appearance along with chlorophyll *a*, carotenoids and phycobiliprotein (Fig. 5e). They are oxygenic photosynthesizing organisms and have unicellular, colonial or filamentous-like forms: the gelatinous sheath of individual cells either remain distinct or fuse into gelatinous matrix; the colonial forms are flat, spherical, elongated, or amorphous; the filaments consist of one or more chains of cells, and each chain is termed as trichome. The blue-green algae have no organelles for locomotion. However, some genera, such as *Oscillatoria*, can move forward, backward or oscillate. The reproduction of Cyanobacteria is vegetative and asexual. Unicellular cyanobacteria divide and reproduce by fusion. Some colonial and filamentous species produce specialized vegetative fragments called hormogonia, which develop into new filaments; other filamentous members form heterocysts, which are larger than vegetative cells. Some genera produce endospores and exospores by internal division of the protoplast. Resting spores called akinetes are also produced by certain species in response to unfavorable conditions (Lee, 2008).

vi. *Phylum Bacillariophyta, Class Fragilariophyceae*

The diatoms are one of the largest and important groups of freshwater or marine organisms. They are oxygenic photosynthetic organisms. Most benthic diatom algae are delicate unicellular organisms or multi-shaped colonies. Many species of diatoms stay connected after cell division and form colonies or long chains in the shape of filaments or ribbons. Sometimes only the tips are connected, so they form a zigzag pattern, or in the shape of stars and fans (Fig. 5f). Their chloroplasts contain pigments such as chlorophylls *a* and *c*, beta-carotene, fucoxanthin, diatoxanthin and diadinoxanthin. Diatom cells are enclosed within a siliceous cell wall (also called frustule) that is coated with a layer of organic material. Diatoms can be divided into two main orders: centric diatoms (Centrales), which are generally radially symmetrical, and pennate diatoms (members of the Pennales), which are bilaterally symmetrical. They reproduce both sexually and asexually (vegetative cell division). Diatoms are the major components of plankton, while many of them are benthic plants.

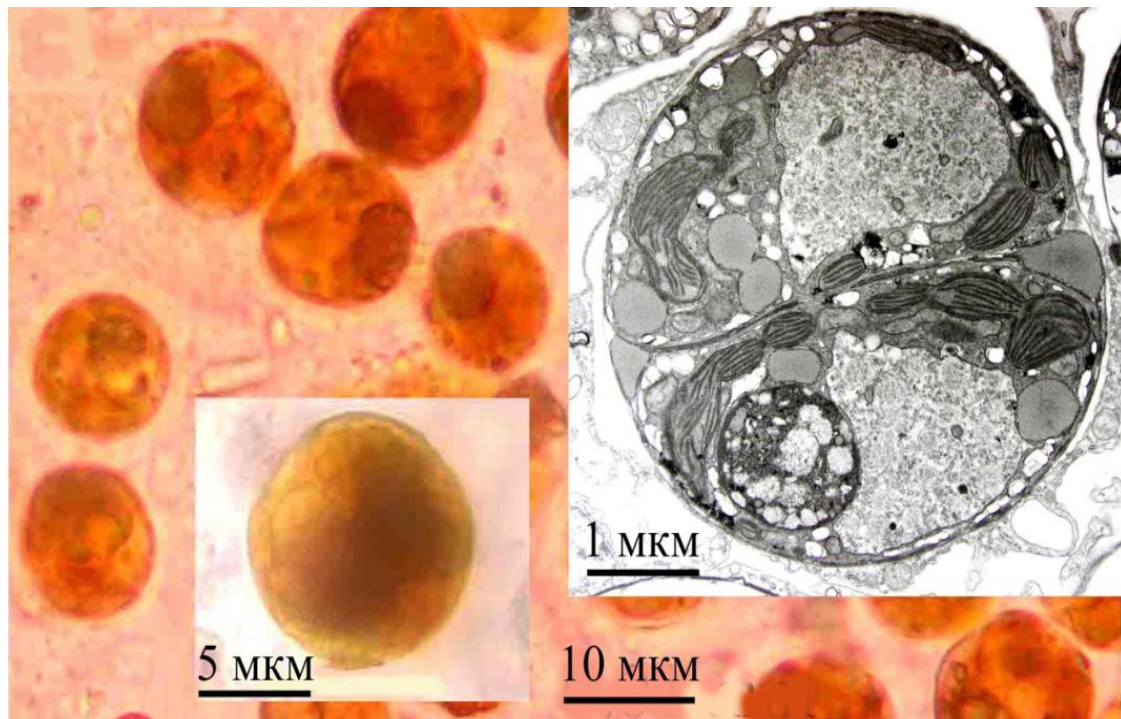
vii. *Phylum Miozoa, Class Dinophyceae*

Zooxanthellae, usually called symbiotic microalgae, mainly live in the endoderm of tropical marine cnidarians (e.g. corals, sea anemones and jellyfish), nudibranchs, sponges, flatworms, hydroids and mollusks (e.g. the giant clam *Tridacna*, some



species of radiolarians and foraminiferans). Generally, zooxanthellae are intracellular symbionts living within a host cell and staying in coccoid (non-motile) stages (6–13  $\mu\text{m}$  in diameter) with thin cell wall (Fig. 6). They are reproduced inside of host cells and transferred from

parents to progenies by sexual or asexual (planulae) products. Distant in systematic state hosts are harbored by various species or even genera of symbionts. Various species of reef-building corals are usually harbored by *Symbiodinium microadriaticum*.



**Fig. 6:** Endosymbiotic algae-zooxanthellae in the tissue of the scleractinian coral *Porites lutea* (Titlyanov, Titlyanova, 2012a). Insert: dividing zooxanthella under an electronic microscope (photographed by Junzo Tsukahara).

*b) Life forms of benthic marine plants*

Marine plants inhabiting coral reefs live in different life forms. For example, epilithic algae inhabit hard substrates, anchored to stones, mollusk shells and dead coral skeletons; endolithic algae settle within hard substrates, e.g., in the skeletons of alive and dead coral colonies, in mollusk or other animals' shells, and in the carbonate base of coral reefs (Fig. 7).



**Fig. 7:** Life forms of benthic marine plants: a – community of epilithic algae (*Asparagopsis taxiformis*, *Padina* sp., *Sargassum* sp. et al.), Cape Bang La (Vietnam), April 2009; b – the green *Halimeda simulans* growing on sandy bottom, Yonaguni Island (Japan), March 2013. Inset: habit, showing bulbous rhizoidal mass binding sand particles; c – fouling algae (crust red algae and the green alga *Ulva papenfussii* on artificial substratum, a car tire, Mot Island (Vietnam), lobster farm, 0.7 m deep, 9 April 2006; d – *Pseudocladophora conchopheria*, overgrowing sea snail, the marine gastropod mollusk *Turbo* (*Lunella*) *coreensis* Récluz, 1853, Amakusa Island (Japan), April 2013. Inset: habit; e – *Ceramium cimbricum*, epiphytic on the red alga *Grateloupia filicina*. Sanya Bay, April 2012; f – the endolithic green alga *Ostreobium quekettii*, living in the skeleton of the scleractinian coral *Porites lutea* (Titlyanov, Titlyanova, 2012); g – two types of blue-green algal films were found on the surface of the holes of dead coral block in the splash zone. *Oscillatoria limosa* (inset) was dominant (Titlyanov et al., 2014); h – the endophytic green alga *Ulvella leptochaete*, living under cuticle of the red alga *Hypnea spinella*. Sanya Bay, Hainan Island, April 2009.

Endolithic algae have two well-defined life forms: colonizing existing cavities within the substrate, and actively penetrating into hard substrates (Fig. 7g, h).

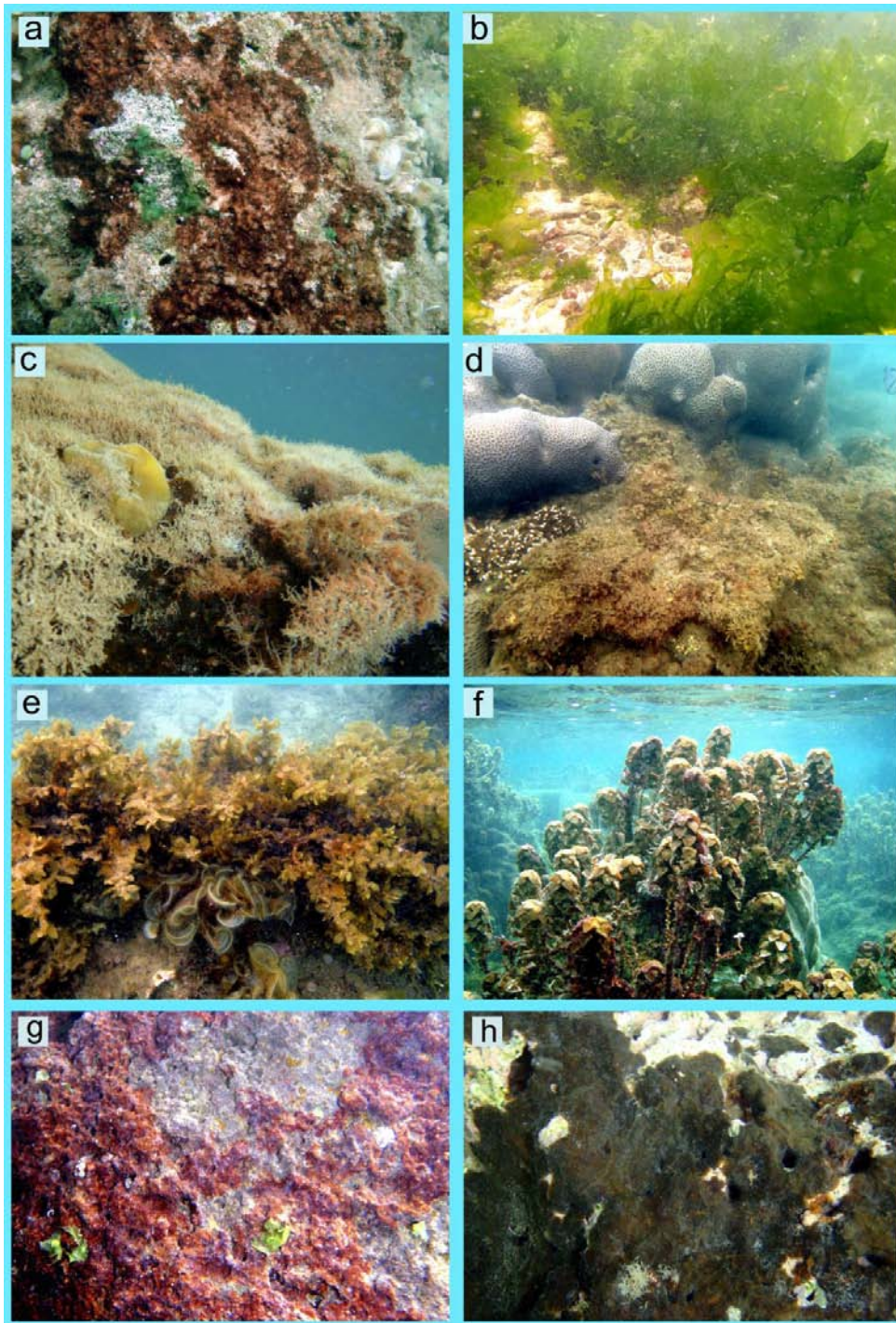
Marine plants can live as symbionts with animals. Among the plants we mentioned above, for example, there are unicellular algae, zooxanthellae, intracellular symbionts of coral endoderm (endosymbionts) (Fig. 6), and symbiotic green and blue-green algae inhabiting skeletons of alive scleractinian corals (ectosymbionts) (Fig. 7f).

Epiphytic algae are attached to thalli of large macroalgae or leaves of seagrasses (Fig. 7 e); epibionts live on hard or soft body surface of animals. Multicellular and unicellular algae can live in intercellular spaces of other plants (endophytes) or be parasitic on marine animals or other species of algae (Fig. 7 h).

c) *The main algal communities on coral reefs*

In reef ecosystem, both short-lived and long-lived algal communities are formed depending on environmental conditions and competitive abilities. As a rule, the short-lived algal communities are fast growing. These communities are commonly formed on newly-formed substrate after natural or anthropogenic catastrophes. It has been shown that the short-lived communities are mostly developed under dramatic changes of temperature, light intensity, salinity, seasons, and tides, especially in the intertidal zones (Fig. 8a). The short-lived communities are mostly blue-green algae, diatoms, filamentous or membranous green algae. They often only consist of one or two algal species. The algal "bloom" is an example of short-lived algae formation on unhealthy coral reefs (Fig. 8b).





**Fig. 8:** The main algal communities on coral reefs: a – temporary community of *Cyanobacteria* in the low intertidal zone, Hainan Island, Luhuitou, April 2012; b – the algal “bloom” of green algae *Ulva* spp., Hainan Island, Luhuitou, March, 2012; c – dense algal turf community composed of dominant species such as *Acanthophora spicifera*, *Palisada papillosa*, *Tolypocladia glomerulata* in the low intertidal zone, Luhuitou, Hainan Island, April 2009; d – algal turf community (predominant species: *Hypnea pannosa*, *Spyridia filamentosa* overgrown with *Gayliella flaccida*) in the upper subtidal zone, Luhuitou, Hainan Island, March 2012; e – macroalgal community with upright foliose morphology (the bidominant community comprised of brown frondose algae, *Sargassum polycystum* and *S. sanyaense*), Hainan Island, Luhuitou, February 2012; f – monodominant community of the brown alga *Turbinaria decurrens* (Dam Trong Islet, south coast of Phu Quoc Island, Vietnam, March 2009; g – monodominant community of the red crust alga *Hildenbrandia rubra* in the upper to middle intertidal zone, Meixia, Hainan Island, March 2012; h – monodominant community of the brown alga *Neoralfsia expansa* in the middle intertidal zone, Luhuitou, Hainan Island, March 2012.

In the subtidal zone, the absence of sharp environmental changes promotes the formation of long-lived communities. In general, the macroalgal community in the subtidal zone includes multiple algal species characterized by several dominant ones. Algal composition and biomass of some species in such community change from season to season. Sometimes, the long-lived communities can also contain only one or two algal species when the conditions are favorable for them.

#### i. Algal Turf Community

Turf, turf-forming, or mat-forming algae occur in coastal waters worldwide, and they have unique importance for coral reefs. However, they are not considered as the most important group of free-living algae regarding the primary production and serving as food source for the herbivorous reef animals (Price, Scott, 1992). Turf algae represent a particular life form of algae, with characteristic biological structure and function. Taxonomically it is a diverse group containing organisms that represent various algal divisions. Members of these different divisions are commonly found on reefs and closely intermixed in multiple assemblages. The turf algae are densely growing, attached to the substratum at numerous points, and their erect branch systems arise from the prostrate axes. Some of the algae form filamentous, cushion-like tufts, bushy clumps, or tangled mats. The general morphological characteristics of turf algae are small size (to about 3 cm in height) at maturity, and slender branches. Juvenile or suppressed individuals of larger macroalgae frequently occur in algal turfs (Fig. 8c, d).

Turf-forming algal communities are resistant to wave actions and very often colonize rocky substrate where the turbidity is higher in the intertidal zone (Carpenter, 1986). Many turf algal species have creeping habit, which makes them well adapted to the intense grazing pressure in 15 coral reef ecosystems. They can regenerate from the remaining branches, creeping axes or basal parts after being destroyed by grazing.

#### ii. Macroalgal communities with upright foliose morphology

Upright macroalgal communities with foliose and fleshy morphology are widely distributed on coral reefs in the low intertidal and upper subtidal zones. These communities usually occupy stony substrata, carbonate base of coral reefs (Fig. 8e, f); some species of them grow on sandy bottom. They are distributed along the border between the low intertidal and upper subtidal zones, forming dense bed comprised of mono- or bidominant communities (e.g. *Sargassum* spp. densely overgrown by epiphytes such as *Chroodactylon ornatum*, *Erythrotrichia carnea*, *Acrochaetium robustum*, *A. microscopicum*, *Colaconema gracile*, *C. hypneae*, *Jania unguolata* f. *brevior*, *Gayliella mazoyeriae*,

*Neosiphonia sphaerocarpa* (Rh), *Kuetzingiella elachistaeformis*, *Sphacelaria novae-hollandiae*, *S. rigidula* (Ph) and *Ulva clathrata* (Ch).

#### iii. Communities of calcareous crust algae

These communities are distributed on hard substrates in the intertidal and upper subtidal zones. The red crust alga *Hildenbrandia rubra* and the brown crust alga *Neoralfsia expansa* are common on flat stones in the upper and middle intertidal zones (Fig. 8g, h).

#### d) Distribution of algae on coral reef and factors regulating their expansion

Both on coral reefs and in other ecosystems marine plants are distributed within the euphotic (epipelagial) zone or illuminated seawater column, where the light conditions allow photosynthetic organisms to complete their life cycles. The light condition in the shallow part of euphotic zone is different and mostly depends on the water transparency. In clean oceanic waters the euphotic zone can extend down to 200 – 250 m deep (Littler et al., 1986).

Light is one of the major factors limiting the expansion of algae into depths. Algae usually do not grow in depths, where the light intensity is smaller than 1% of PARS, i.e., approximately 10 – 15  $\mu\text{E}/(\text{m}^2 \text{ s})$  if measured at the noon of a sunny day (Titlyanov, 1999). However, red coralline algae of the genus *Lithothamnion* are widely distributed in deeper sites in subtropical areas, where the illumination level ranges within 0.05 – 0.1% of PARS (Molinier, 1960; Lang, 1974; Littler et al., 1986).

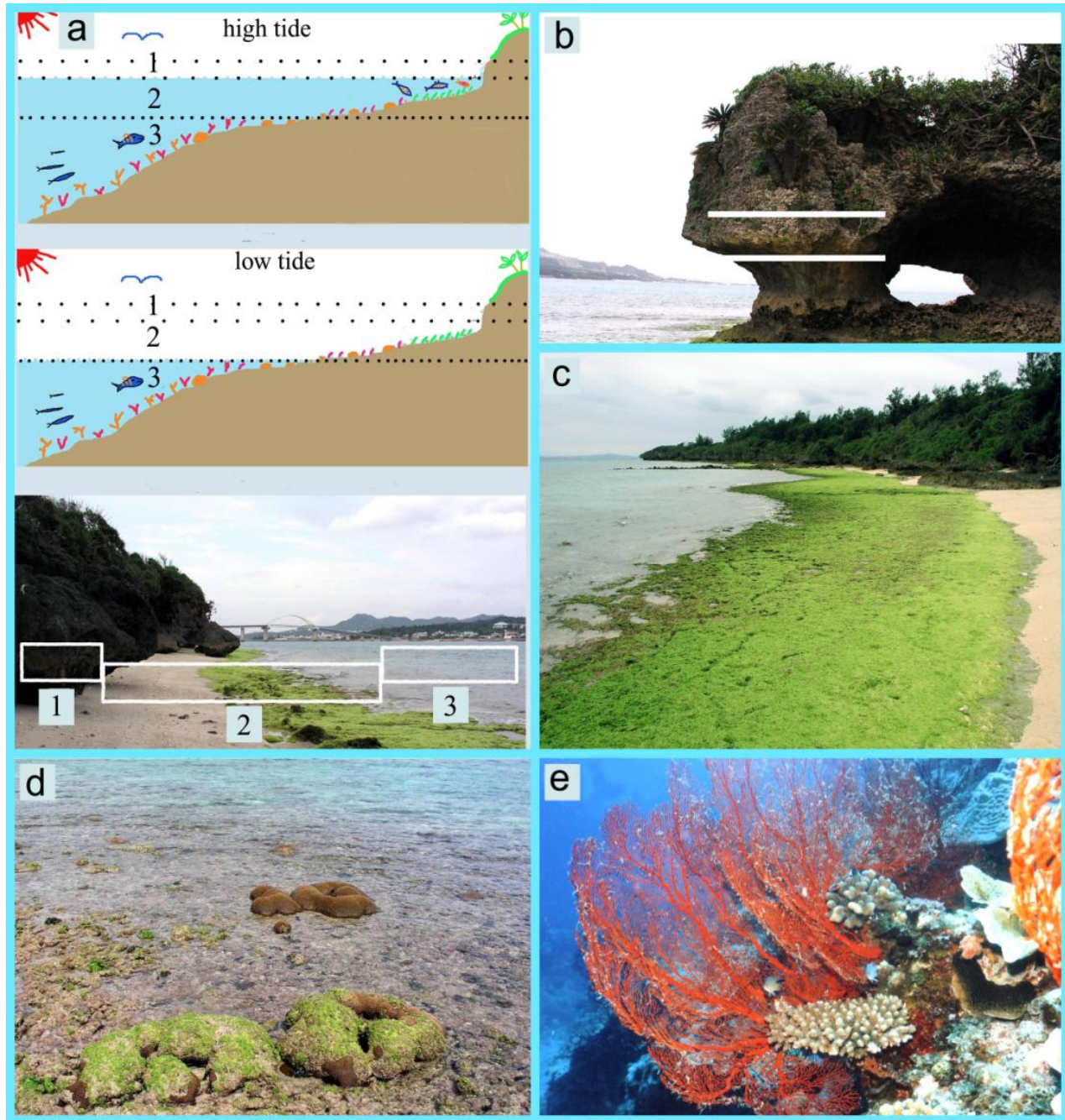
If interspecies competition is absent, almost all algae can survive with the light intensity ranging from 1 to 100% of PARS, despite some species are vulnerable to ultraviolet so that they cannot live in the intertidal area under the direct sunlight (Fong, Paul, 2011).

Unlike higher plants, marine algae can adapt to an extremely wide range of light intensity. The process of adaptation involves the initiation of certain mechanisms that is associated with numerous adaptive responses. For example, the mechanisms, such as maximization of light absorption, efficient utilization of absorbed light and saving consumption of nutrients, can help plants adapt to extremely low light level. Maximization of the light absorption will mainly lead to the accumulation of photosynthetic pigments. Efficient utilization of absorbed light can boost the quantum output of photosynthesis in shaded plants. Saving consumption of reserve and structural substances is achieved through slowing down the respiration and the excretion of assimilated substances (Titlyanov, 1999).

Depending on their physiological capabilities, marine plants can inhabit in three different ecological reef zones (Fig. 9). The first zone is located at the border between the sea and the land, which is supratidal fringe

or splash zone (Fig. 9a, b). Most of the time, this area is above water level and is only moistened by strong storms or extremely high tides. The marine plants in

supratidal area usually settle in shaded rocky crevices, small cavities (grottoes) on vertical walls, or under the rocky ledges.



**Fig. 9:** Different ecological reef zones (coral reef of Sesoko Island, Okinawa Prefecture, Japan: a – scheme of the distribution of tidal zones (1 – supratidal fringe or the splash zone, 2 – the intertidal or littoral zone, 3 – the upper subtidal zone); b – supratidal fringe; c, d – the intertidal zone; e – the upper subtidal zone.

The second ecological zone is the intertidal or littoral area. Marine plants inhabiting this zone are dipped in water during high tides and drying up during low tides. Algae living in intertidal pools (depressions in rocky or boulder-blocky grounds) are permanently covered by water (Fig. 9a, c, d).

The third zone is the upper subtidal zone. Marine plants and animals in this zone mostly remain submerged (Fig. 9e).

The environmental conditions are strikingly different in different zones. Temperature conditions are especially varying in the supratidal fringe and upper

intertidal zone. In these zones, algae are often exposed to air and can even desiccate without losing viability, where as subtidal algal species are less tolerant to desiccation. During the rainy seasons the algae are prone to desalination. The unfavorable conditions for algae in supratidal zone are also harmful for intertidal algae during low tides. Supratidal and intertidal organisms are adapted to rapid and dramatic fluctuations of temperature, illumination, salinity, humidity, pH, oxygen concentration, and carbon dioxide concentration (Fong, Paul, 2011).

The algae in the subtidal zone live under more favorable and more stable conditions. In tropical reef ecosystems, the water temperature usually ranges from 24°C to 30°C, and its fluctuations related to different depths and winter/summer transitions are very small. Therefore, the temperature, in all likelihood, does not belong to factors that limit the distribution of marine plants in the subtidal zone.

The salinity of water in coastal ecosystems of continents and large islands depends on the amount of precipitation and ranges within 24 – 34‰. Due to the natural resistance of marine plants to salinity fluctuations, temporary small changes of water salinity on coral reefs exert almost no effect on their viability and the structure of their communities. The desalination of seawater that happens close to estuaries of large rivers affects symbiotic corals much more than marine algae. The corals could suffer from osmotic shock and lose their zooxanthellae partially or entirely. If they cannot recover after the shock, they will die and then get occupied by epilithic algae (Titlyanov et al., 2000, 2008a; Diaz-Pulido, McCook, 2002).

Dissolved inorganic nitrogen (ammonium, nitrates and nitrites) is one of the major factors that ensure the growth of algae on coral reefs. The concentrations of mineral nutrients in the water around coral reefs are usually greater than that in neighboring ocean waters, but are lower than that in coastal estuaries and lagoons (Crossland, 1983; D'Elia, Wiebe, 1990; Szmant, 2002; Atkinson, Falter, 2003; Atkinson, 2011). Nitrogen-fixing blue-green algae can increase nitrogen concentration and benefit the balance of nitrogen-containing substances in coral reef ecosystem (McClanahan et al., 2007). On coral reefs there is enough phosphorus due to its turnover within the ecosystem (Atkinson, 2011). However, some studies showed that phosphorus can be removed from the ecosystems as a result of calcium binding to phosphate ions; if so, the algae will also suffer from a phosphorus shortage (Fong et al., 1993).

The upper subtidal zone is inhabited by algae that are most tolerant to high water turbulence. For example, gulfweeds and coralline algae can grow and withstand surf actions in the surf areas of coral reef, on the outer side of reef flat, and within the reef crest. In dense communities of fleshy algae (e.g., *Sargassum* spp.), as well as in algal turf, the mild water turbulence

helps the inflow of nutrients to the plants (Sousa, 1984; Hurd, 2000).

The ecological zones described above vary a lot in the effects of certain biotic factors on marine algae. For example, in the supratidal zone the grazing of algae by fish and invertebrates is almost excluded. However, in the intertidal area, grazing is determined by tides and water turbulence associated with tidal currents and surf. Grazing by herbivorous animals is an important factor of succession control for algal communities. It regulates the rate of biomass accumulation, frequency and intensity of reproduction in individual plant. The major herbivorous animals on coral reefs are fishes, sea urchins and gastropod mollusks (Glynn, Enochs, 2011; Montgomery, 2011).

On a healthy coral reef with unpolluted water, the competition for substrate and resources utilization between marine algae and corals is insignificant and not so important for development of plants and coral communities. On these reefs, the percentage of bottom covered by coral is around 80 – 90% without changing for years. However, after damages to coral reefs, especially after massive mortality of scleractinian corals, the competition between algae and corals for newly available substrates becomes crucial for restoring and maintaining the reef ecosystem. Dead and damaged coral colonies are occupied by benthic micro- and macroalgae during the first year after the ecological disaster. The coverage of carbonate reef base by living corals decreases, whereas the degree of algal coverage rises up (McCook et al., 2001; Titlyanov, Titlyanova, 2008; Chadwick, Morrow, 2011).

Littler and Littler (1984) came up with a hypothesis that the distribution of corals and different algal communities on coral reefs depends on the effects of major abiotic and biotic factors (Fig. 10). According to this hypothesis, hermatypic corals containing zooxanthellae usually successively occupy substrate and space under the conditions of intensive grazing of macrobenthic algae by animals, moderate levels of wave actions, and low concentration of nutrients in seawater. Coralline algae will dominate in areas with moderate or strong grazing of non-calcified algae, strong wave effects, and moderate or high concentration of nutrients. Communities of algal turf develop under low concentration of mineral nutrients and small level of grazing. The beds of large fleshy and foliaceous (frondose) macrophytes occupy ecological niches that are rich in nutrients and free of pressure from herbivorous animals (Littler, Littler, 1984). Fig. 10. Modified Littler and Littler (1984).

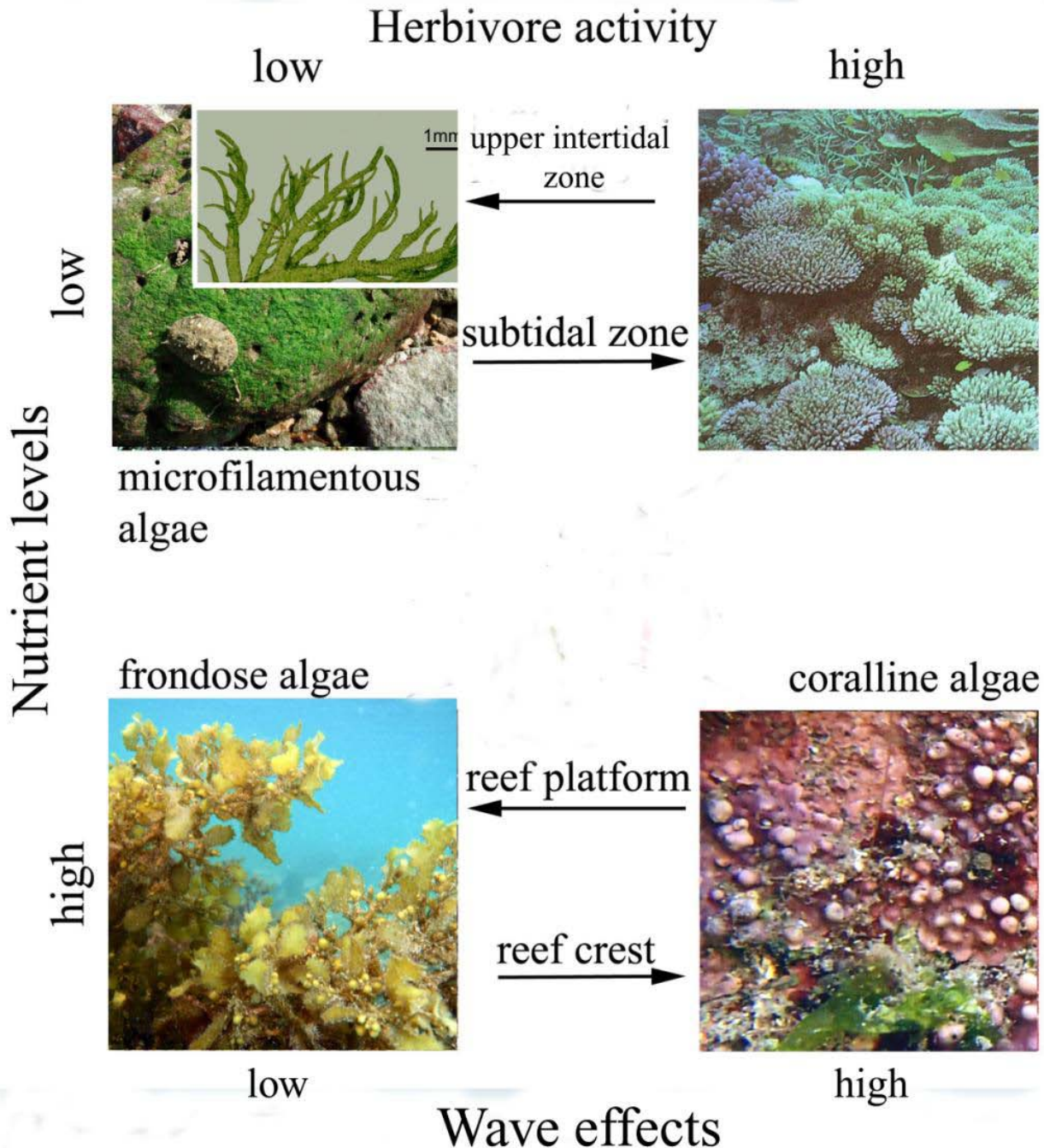


Fig. 10: Modified Littler and Littler (1984) representation of the relative dominance paradigm

e) *The role of marine plants in coral reef ecosystem*

i. *Primary production of organic matter and its turnover*

Coral reefs are one of the most diverse and productive ecosystems on Earth. They form heterogenous habitats that serve as important sources of primary production within tropical marine environments (Odum, Odum, 1955; Connell, 1978). The total coral reef coverage amounts to 600,000 km<sup>2</sup>, which is about 0.17% of the ocean surface. The gross

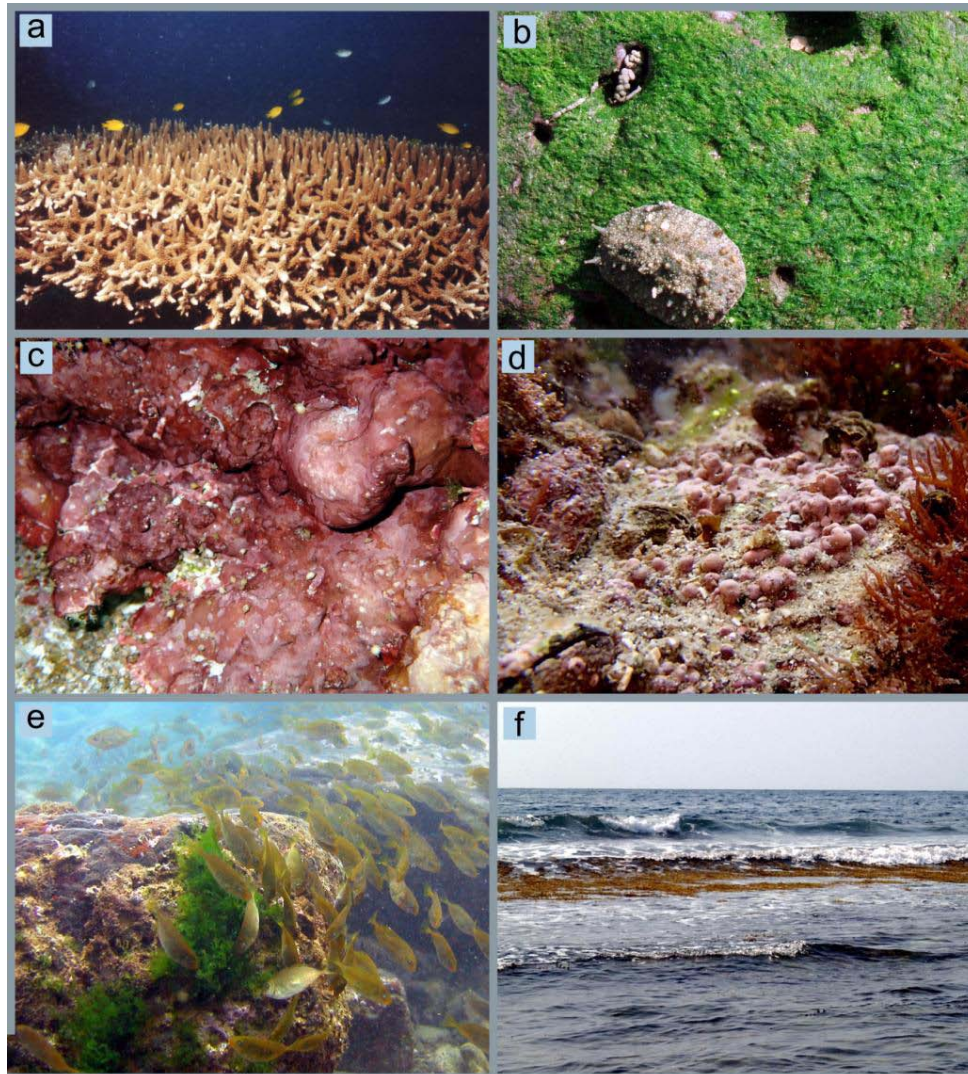
fixation of carbon by coral reefs is  $20 \times 10^{12}$  g per year, 15% of which is consumed by the reef system, 10% is used by human, and the remaining 75% is exported into adjacent areas in the ocean (Crossland et al., 1991).

The massive production of coral reefs is determined by several factors: efficient absorption of incident light by reef photosynthesizing organisms, complete structure of coral reefs, and high degree photoacclimation of reef photosynthesizing organisms



(Titlyanov, 1991; Titlyanov, Titlyanova, 2002a, b; Dubinsky, Falkowski, 2011). On coral reefs, the illuminated bottom surface in the upper subtidal zone is almost entirely covered by photosynthesizing organisms, and the most illuminated areas are occupied by the most productive organisms, the scleractinian corals (Fig. 11a). The primary production of the

scleractinian corals is made up by the production of endosymbiotic unicellular algae, zooxanthellae (Fig. 6), and ectosymbiotic green and blue-green algae (Fig. 7f). The communities of finely lamellar and finely filiform macroalgae are also very productive (Fig. 11b), which occupy brightly illuminated ecological niches in the intertidal area.



**Fig. 11:** The role of marine plants in coral reef ecosystem: a - on healthy reef the illuminated bottom surface in the upper subtidal zone is almost entirely covered by the most productive photosynthesizing organisms, the scleractinian corals; b - in the intertidal zone the most illuminated areas are occupied by very dense communities of fine filamentous, high productive green algae (for example, temporary monodominant community of the green alga *Ulva clathrata* in the upper intertidal zone of the Luhuitou coral reef in Sanya Bay, October 2008; c - the shaded deep-sea areas and strongly shaded grottoes of reefs are mostly inhabited by low-productive long-lived red coralline algae (e.g. the shadowed niche in the subtidal zone of coral reef of Yonaguni Island in the Ryukyu Archipelago chain (Okinawa Prefecture, Japan), March 2013; d - the communities of calcareous crustose algae connect together the neighboring areas of carbonate reef base, dead skeletons of colonies, they protect the reef from erosion and build a new carbonate layer on the base of the coral reefs. The red calcareous alga *Lithophyllum okamurae* on damaged coral reef of Yonaguni Island (Ryukyu Archipelago, Japan, March 2013); e - marine macrobenthic algae are the major food of herbivorous animals inhabiting coral reefs, Vietnam, Nha Trang Bay; f - frondose algae of the genera *Sargassum*, inhabiting the lower intertidal zone and the uppermost part of the subtidal zone and forming extensive beds protect reefs from wave action and erosion.

Planktonic microalgae and symbiotic algae of sponges, soft corals and mollusks are also producers of organic substance on coral reefs, but their contribution is insignificant (Crossland et al., 1991; Fong, Paul, 2011). The shaded deep-sea areas of reefs are mostly inhabited by low-productive long-lived red coralline algae (Fig. 11c). On undamaged coral reefs (percentage of substrate covered by corals exceeds 50%); the major primary production is provided by unicellular symbiotic algae, zooxanthellae. While on damage coral reefs, macrophytes can become the major producers (Titlyanov, Titlyanova, 2008; Stambler, 2011).

Dying algae are washed up on the coast or gravitate to the bottom, where they are held among patch reefs. In both cases they become food for animals (consumers) and bacteria (reducers). Bacteria can transform organic substance into inorganic compounds, which increase the concentrations of dissolved nitrogen and phosphorus (especially in lagoons) up to the level that is enough to support high productivity of marine plants (Fong, Paul, 2011).

#### ii. Reef-Builders

The communities of calcareous crustose red algae of the genera *Porolithon*, *Peyssonnelia*, *Lithothamnion* and *Lithophyllum* are reef-builders. They connect together the neighboring areas of carbonate reef base, dead skeletons of colonies, coral pebbles, cement carbonate sand, and other sediments located in-between coral colonies. Therefore, they protect the reef from erosion and build a new carbonate layer on the base of the coral reefs (Fig. 11d). Calcium carbonate is deposited within the tissues of crustose calcareous algae at the rate of more than 10 kg CaCO<sub>3</sub>/m<sup>2</sup> per year. In the subtidal zone, these algae can build up carbonate structures down to 50 – 100 m deep (Molinier, 1960; Lang, 1974; Littler et al., 1986; Chisholm, 2003). The communities of large calcified green algae (the genera *Halimeda* and *Udotea*) and red algae (the genera *Amphiroa* and *Galaxaura*) also produce calcium carbonate (Macintyre et al., 1987; Hine et al., 1988, Marshall, Davies, 1988, Roberts et al., 1988, Hillis, 1997, Merceron et al., 2007; Nelson, 2009). The remnants of thalli from these algae transform into carbonate sands, which fill up gaps between coral colonies on the bottom and serve as building materials for coral reefs.

#### iii. Nitrogen Fixation

An important function of marine plants on the reef is nitrogen fixation. It is performed by cyanobacteria (members of the genera *Lyngbya*, *Oscillatoria*, *Calothrix*, *Anabaena*, *Entophysalis*, *Nodularia*, etc.) inhabiting soft sediments in-between coral colonies. They live also on stones and dead coral colonies, being epiphytic on seagrasses and endolithic inside of coral skeletons (Fig. 5e). The rate of nitrogen fixation by blue-green algae

depends on factors as light intensity, availability of dissolved nutrients and water temperature (Bergman et al., 1997; Welsh et al., 2000; Dong et al., 2002a, b, 2006, 2008; Lugomela, Bergman, 2002; Hamisi et al., 2004).

#### iv. Marine plants are the initial link of food chains

Marine macrobenthic algae and epiphytic microalgae are the major food of herbivorous animals inhabiting coral reefs (Fig. 11e). Experiments have shown that herbivorous animals can consume up to 100% of daily production of macroalgae during one day (Carpenter, 1986; Duffy, Hay, 1990; Hay, 1997; Burkepile, Hay, 2006; Hughes et al., 2007). Fishes from families like *Blenniidae*, *Kyphosidae* and *Siganidae* are selectively grazing filamentous and fleshy algae from turf community, thus helping calcareous coralline and other crustose algae to grow.

Some fish species are grazing green algae of the genera *Cladophora*, *Enteromorpha* and *Ulva*; other fishes are foraging on brown algae of the genera *Sargassum* and *Dictyota* (Hay, 1997). Besides fishes, marine algae are also eaten by sea urchins, mollusks, crabs and amphipods. Seagrasses are consumed only by animals that can digest cellulose, such as sea green turtles, manatees and dugongs.

#### v. Communities of marine plants provide environment for marine animals

Communities of algal turf (especially in the subtidal zone), large densely branching gulfweeds, and seagrasses provide good refuges for fish, crustaceans and mollusks, as well as their larvae. Articulated coralline algae provide refuges for many species of small invertebrates (Nelson, 2009; Fong, Paul, 2011). Some scientists distinguish specific fauna of these algal communities (Kelaher, 2002; Kelaher et al., 2004; Chapman et al., 2005; Liuzzi, Gappa, 2008).

Large macrophytes and crustose calcareous algae are good substrate for some sessile marine animals to settle down, like hydroids, spirorbids, polychaetes, bryozoans and foraminiferans. Coralline algae serve as substrate for scleractinian coral planulae (Vermeij et al., 2009; Ritson-Williams et al., 2010). Coralline algae are utilized by marine farms specialized on abalone (*Haliotis* spp.) growing, serving as a substrate for settlement and development of abalone larvae (Morse, Morse, 1991).

Additionally, old thalli of macroalgae and leaves of seagrasses are populated by numerous epiphytic and endophytic algae, as well as saprophytic marine fungi. Therefore, marine plants ensure one of the major conditions for coral reef to exist, its high biodiversity.

#### vi. Protection of reefs against deleterious effects from surf

Coralline algae are highly resistant to surf. They settle on reef crest and develop a stout ridge that functions like a breakwater, which protects delicate algal forms and animals inhabiting the upper part of reef

slope from damages. The coriaceous and frondose algae of the genera *Turbinaria* and *Sargassum* (Fig. 11f), inhabiting the lower intertidal zone and the uppermost part of the subtidal zone, also play the similar roles (Littler, Littler, 1988).

### III. REEFS UNDER DISTURBANCES

#### a) *Natural and semi-natural disturbances*

Coral reef ecosystems are susceptible to natural and semi-natural disturbances which, if their magnitude is such that the threshold of system tolerance/resilience is exceeded, may lead to major changes in ecosystem functioning (Steinberg, 2012). Throughout the evolutionary history of coral reefs, they have been exposed to various forms of natural catastrophes including super-typhoons, tsunamis, volcanic activities, sea temperature fluctuations and sea level changes that have led to major shifts in reef communities.

#### b) *Possible role of marine plants in damages, mortality or recovery of coral reefs*

Severe physical disturbances such as typhoons/hurricanes/cyclones and tsunamis cause extensive damages (e.g., fragmentation and dislodgment) in coral colonies (Woodley et al. 1981; Glynn 1990). Death of hard corals leads to the loss of architectural complexity and reef flattening through the collapse of coral skeletons. As such, disturbance is a well-known modifier of reef seascapes. In marine systems stormy conditions may not only remove or bury subtidal organisms but they may also help form new substrate patches. In the wake of a strong disturbance, newly formed substrates appear as bare rocks and banks formed of sand and coral fragments, with dead/damaged coral colonies providing space for sessile organisms (Rogers et al., 2008; Massel, Done, 1993; Trenberth, Shea, 2006; Manzello et al., 2007; Alvarez-Filip et al., 2009).

Coral bleaching is often caused by unusually high sea temperatures (>30°C) combined with periods of slack wind, calm seas, cloudiness, high solar radiation, and in some areas, reduced salinity due to extreme weather events, such as typhoons, storm surges, storms, or floods. Bleaching leads to reduced photosynthesis, a tissue growth, regeneration, calcification and subsequently to the death of corals (Lesser et al., 2007). On some reefs, up to 100% of corals died within few months after a bleaching event (Baker et al., 2008). Elevated seawater temperatures that result in coral bleaching may also negatively affect algae. It is well known that some Corallinaceae species (e.g., *Corallina officinalis*) are bleached (Latham, 2008), but to date there has been no documented record of mass bleaching of seaweeds or their destruction due to a bleaching episode.

The speed of recovery often depends on the severity of bleaching disturbance, and on the amount of coral cover remaining after the disturbance (Loch et al., 2002; Stobart et al., 2005; Guzman, Cortes, 2007; Glynn et al., 2011). A decline in live coral cover may not follow a single bleaching episode but often depends on accompanying coral diseases, *Acanthaster* predation and the occurrence of repeated bleaching episodes. Eventually, bioerosion and mechanical fragmentation of reef materials generate unstable rubble- and sand-substrates which are unfavorable for coral recruitment (Szmant, 2002; Baker et al., 2008; Rogers et al., 2008). Patterns of loss and recovery in coral cover at several eastern Pacific sites ranged from total elimination to total recovery with periods spanning 10 – 28 years (Wellington, Glynn, 2007). Recovery of undisturbed and slightly affected coral reefs was recorded at annual rates of 1 – 10%, while gradual decline in live coral cover ensued at sites experiencing severe anthropogenic stresses (Connell et al., 1997; McClanahan et al., 2007; Baker et al., 2008).

Severe natural catastrophes on coral reefs result in the formation of new substrata, changes in the relative abundances of surviving hermatypic corals and the dominance of non-coral taxa associated with reef assemblages. The fate of a damaged reef depends on factors such as the degree of coral reef damage, the presence of coral and non-coral taxa on the reef and sources of planulae supply for recolonization and restoration of coral populations (Harrison, 2011). A damaged coral reef may completely return to its initial state or attain a changed state with the predominance of other species and forms of corals or turn to non-coral taxa reef (Baker et al., 2008; Titlyanov, Titlyanova, 2012b) (Fig 12).

## Possible fate of the damaged coral reef

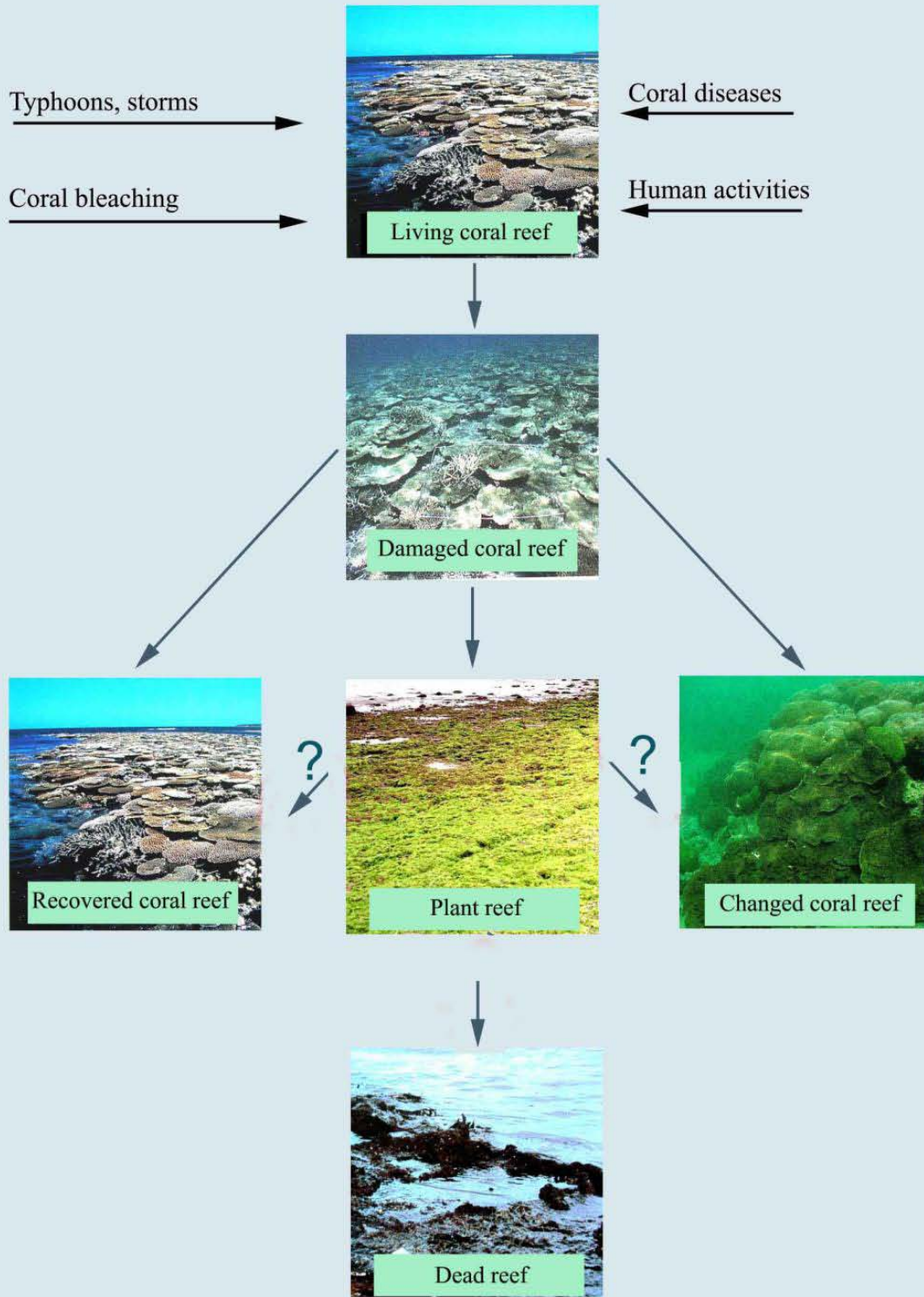


Fig. 12: Possible fate of the damaged coral reef

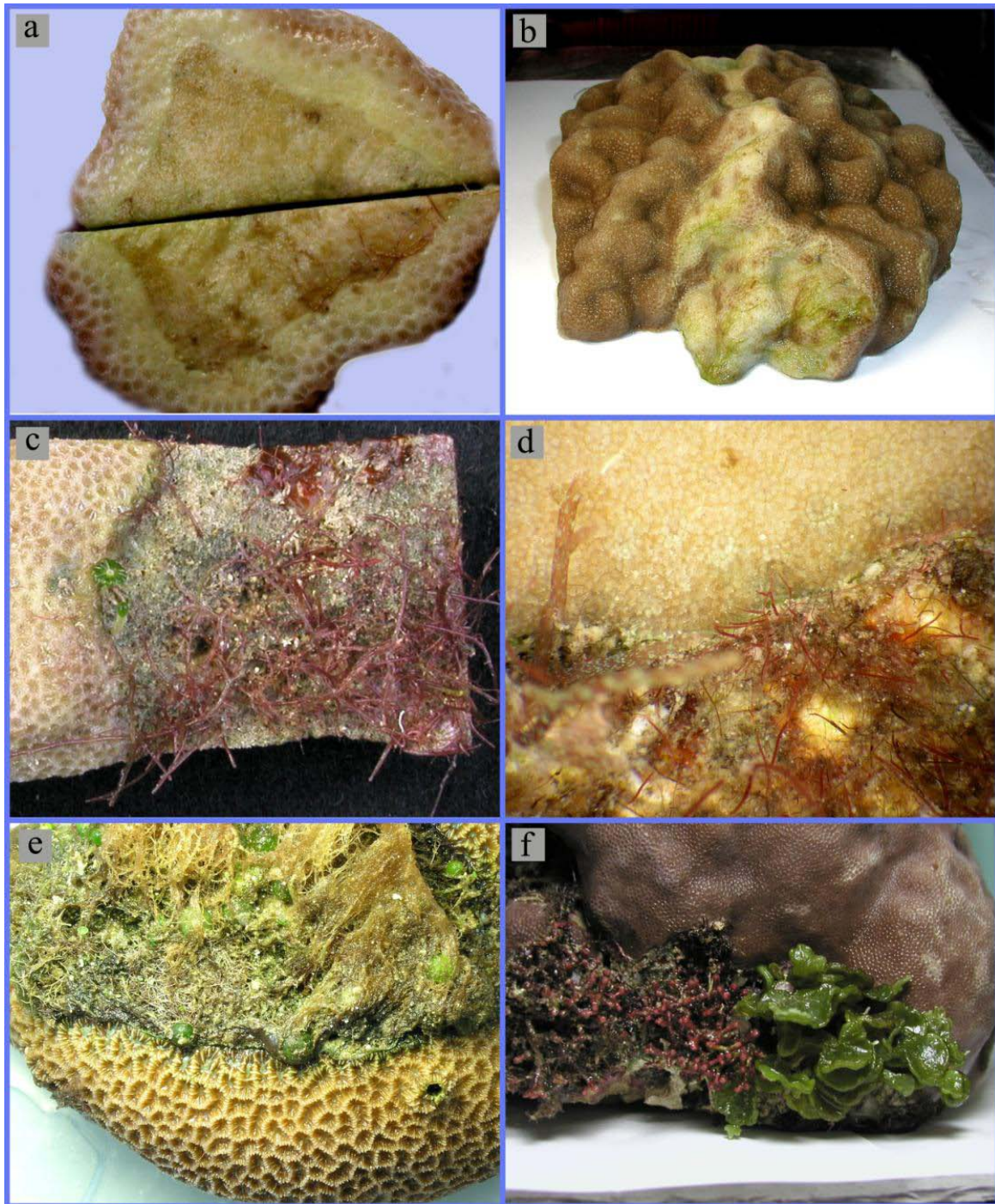
i. *Possible fate of the damaged coral reef*

A number of studies reported that severely damaged coral reefs were subsequently transformed into a seaweed-dominated state ("phase shifts") (Done, 1992; Knowlton, 1992; McManus, Polsenberg, 2004). Bruno et al., (2009), however, indicated that the replacement of corals by macroalgae as dominant benthos was less common and less geographically extensive than assumed, based on a meta-analysis of 3581 quantitative surveys conducted in 1996 - 2006 involving 1851 reefs around the world. These give credence to the view that such a "phase shift" represents a temporary state of coral reefs on their way to recovery to an initial or changed state (Titlyanov, Titlyanova, 2012b).

ii. *Colonization of newly formed substrates*

Newly formed substrates (after bleaching events, etc.) made of dead or damaged colonies of hermatypic corals are rapidly colonized by sessile organisms such as sponges, hydroids, gorgonians as well as various algae. As was shown by field and laboratory studies (Diaz-Pulido, McCook, 2002, 2003, 2004; Diaz-Pulido et al., 2007; Mumby, 2009; Chadwick, Morrow, 2011), microscopic diatoms, fine filamentous blue-green and green algae, tunicates, foraminifera, small-sized polychaetes are the first colonizers on both dead and wounded coral colonies.

Studies involving mechanically damaged colonies (with various types of injuries) and dead coral debris (pebbles) of massive and branched corals (Titlyanov et al., 2005, 2006, 2008b) showed that the injuries and pebbles were immediately overgrown by algae and cyanobacteria. After the first month of colonization, algae occupied 1 – 7% of the injured surfaces and 1 – 4% of the surface of pebbles. Algae settled only on the skeleton surfaces not covered by live coral tissue. The first settlers were microscopic, micro-filamentous, fine filamentous and filamentous-tubular forms (Fig. 13a, b). By the third month, algal cover significantly increased to 30 – 50% on the injuries and to 25 – 60% on coral pebbles. The composition of dominant species on the lesions had changed, but ephemeral algae still dominated (Fig. 13c, d). During the 6 – 8 months of the experimental period, the projected cover of algae amounted to 80 – 100% on the lesions and 60 – 90% on coral pebbles, where an algal turf community with the dominance of ephemeral as well as long-lived species was formed (Fig. 13e, f). Algal communities on new substrata lasted from 6 – 8 months (algal turfs) to some years and subsequently species composition, biomass and density of these communities changed seasonally and depending on competitive abilities of different settlers.



**Fig. 13:** Polyp regeneration and algal settlement onto inflicted damages on coral colonies: a, b – algal settlement onto inflicted damages on *Porites lutea* colonies, after the first month of colonization, c, d – algal settlement onto inflicted damages on *Porites lutea* (c) and *Porites cylindrical*; d – colonies after three months of the colonization; e, f – the formation of algal turf on inflicted damages on *Platygyra verweyi* (e) and *Porites lutea* colonies (f) after 6 – 8 months of the colonization

### iii. Coral-algal relations

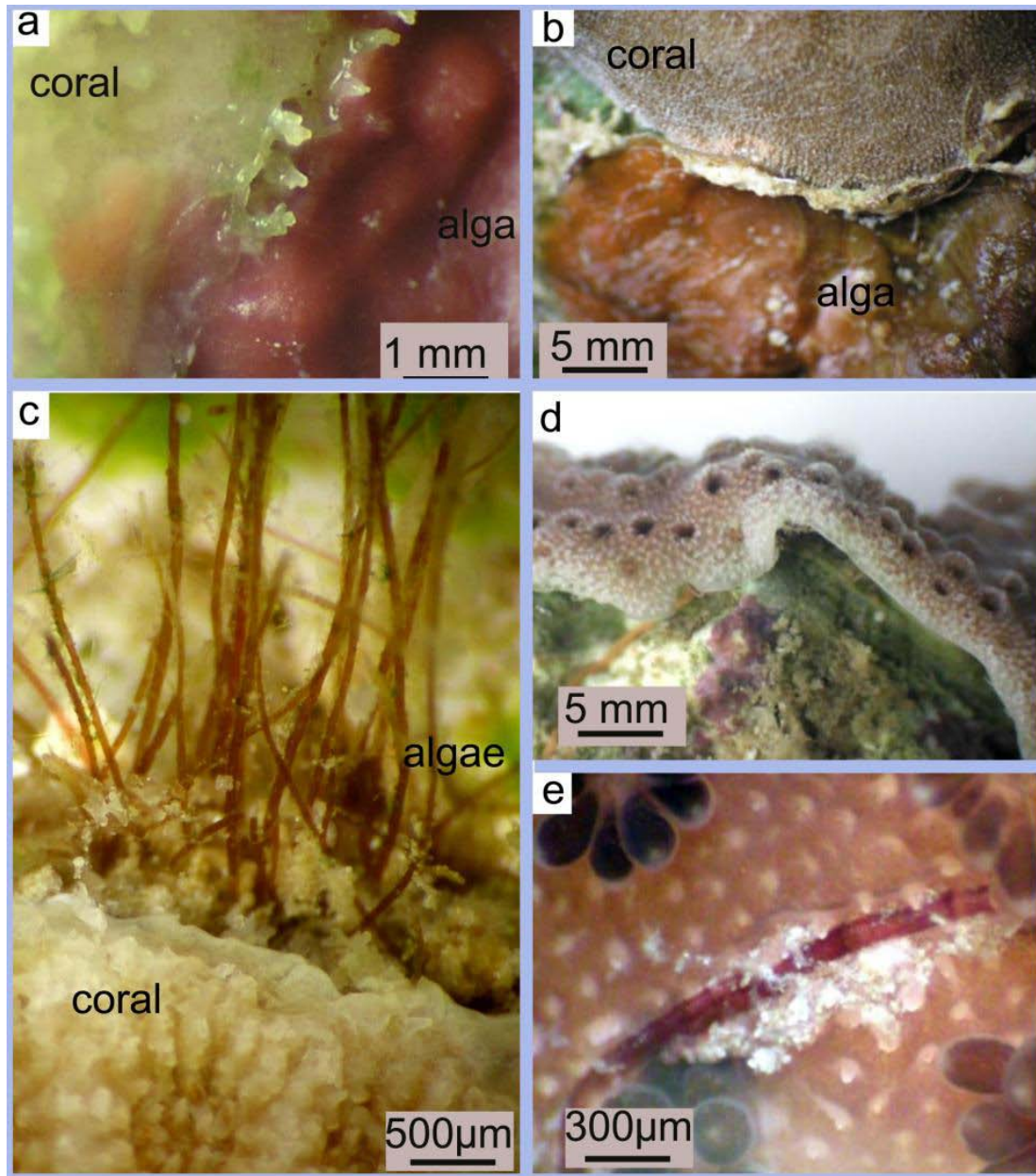
As discussed above, algae occupy newly-released substrata within some months and are involved in competitive relationships with survived and newly settled corals and other invertebrates. Successional interactions begin from the first stages of algal

community colonization and complete with the transition of a reef into one of the stable states where either hermatypic corals or algae become dominant. With the transition of a damaged reef into a stable state, competitive relationships are generally replaced by symbiotic (mutualistic) relationships that contribute to

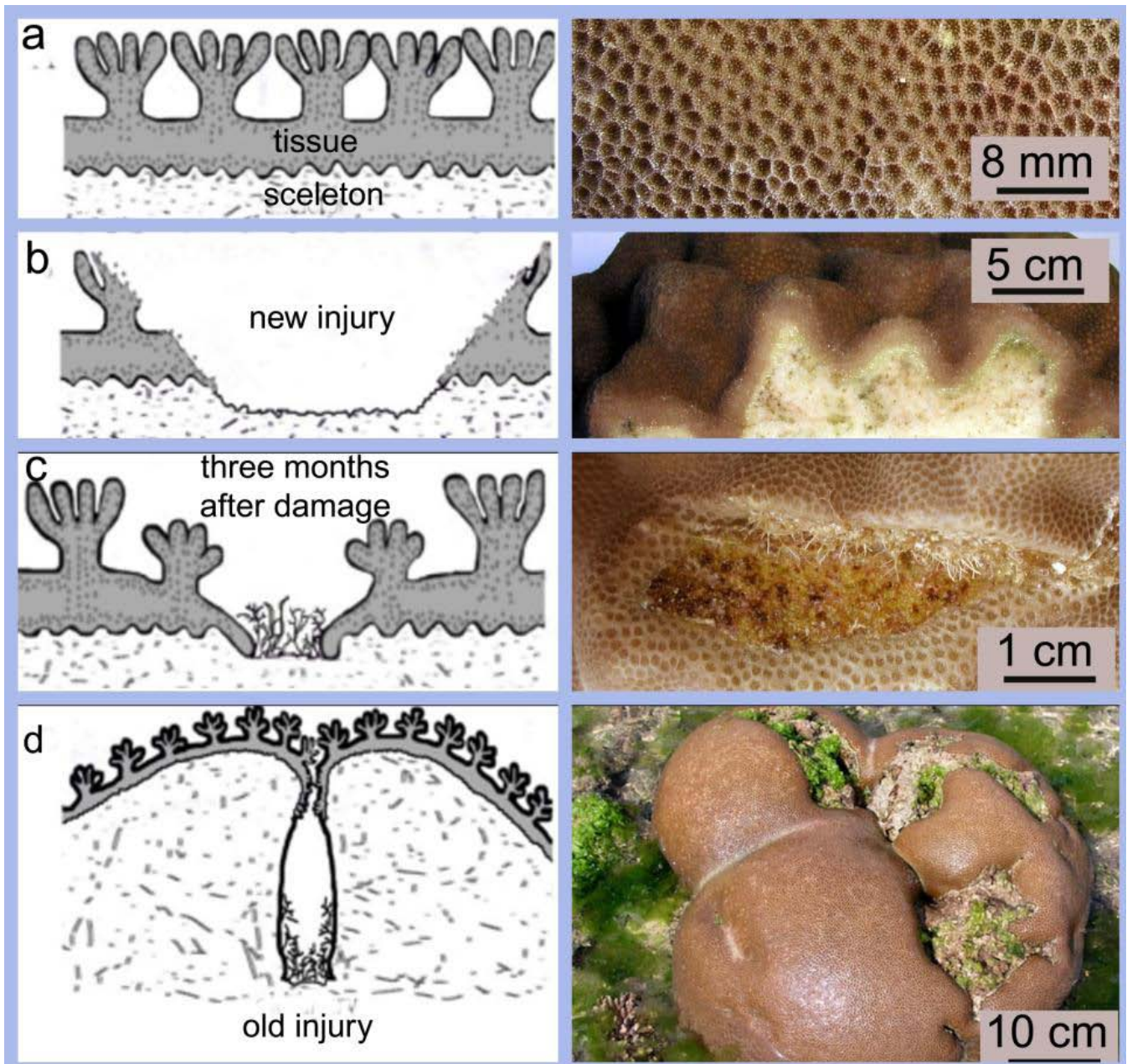
the maintenance of ecosystem stability or homeostasis (Petraitis, Dudgeon, 2004).

At early stages of colonization, competition between corals and algae is characterized by overgrowth of competitors and allelopathic influences (McCook et al., 2001), where coral polyps that survived a catastrophe tend to be competitively superior (Titlyanov, Titlyanova, 2008). In an experiment on the regeneration of adult colonies of *Porites lutea* and *P.*

*cylindrica* from artificially-inflicted injuries, coral polyps were able to overgrow more than 100 algal taxa (but not toxic blue-green algae such as *Lyngbya semiplena* and *L. majuscula*). Injuries up to 20 cm<sup>2</sup> in area healed within 6 months, i.e. the algal-turf community formation was not a serious impediment to the recovery of damaged corals, although the rate of recovery slowed down (Figs. 14, 15, 16).

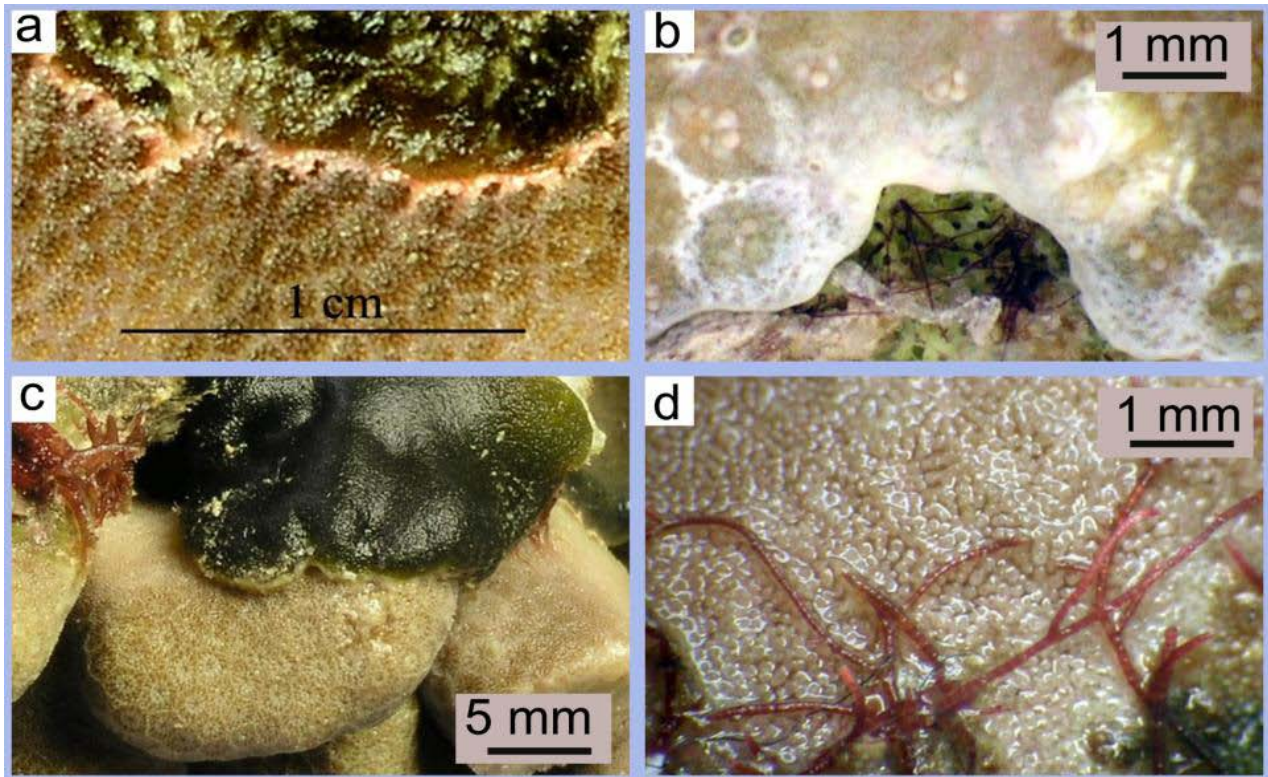


**Fig.14:** Various ways of overgrowth of algae by corals under moderate light (20 – 30% PAR0) (Titlyanov, Titlyanova, 2008; Titlyanov et al., 2009): a – coral-algal competition for substratum of the coral *Porites lutea* and the red crust alga *Peyssonnelia conchicola*. Polyps overgrew algal thalli with thin, transparent, azooxanthellate tissue (50 – 200 μm wide); b – overgrowth of the brown alga *Lobophora variegata* by the coral *Porites lutea* (by the release of mucus by the coral); c – coral expansion on algal turf (coral tissue produce skeletal crystals); d – overgrowth of blue-green and red crust algae by encrusting coral (forming canopy above algae); e – entombing into skeleton of the red alga *Centroceras clavulatum* by the coral *Acropora* sp. (Titlyanov, Titlyanova, 2008).



**Fig.15:** Scheme of stage-by-stage regeneration of damaged colony of the hermatypic coral *Porites lutea*: a – polyps of healthy (undamaged) coral; b – injury (one week old); c – injury (4 month old) overgrowing by polyps; d – old wound (4 years old) of recovering coral colony (Titlyanov, Titlyanova, 2008).





**Fig. 16:** Allelopathic influence of Cyanobacteria on corals and overgrowth of corals by macroalgae: a – The coral *Porites lutea* under allelopathic contact with the blue-green alga *Lyngbya semiplena* (coral changing growth form in upright direction); b – bleaching and inhibition of polyps' growth of the coral *Porites lutea* under allelopathic contact with the blue-green alga *Lyngbya majuscula*; c – overgrowth of the coral *Porites cylindrica* by the green alga *Codium arabica* under low light (2–1% PAR<sub>0</sub>); d – overgrowth of the coral *Porites lutea* by the red alga *Centroceras clavulatum* under low light (Titlyanov et al., 2005).

After the formation of algal communities on vacant substrata, competition between coral colonies and algal settlers continue with different modes of competition being involved. These include direct physical interactions such as overgrowing, overtopping, smothering (McCook et al., 2001; Box, Mumby, 2007; Titlyanov, Titlyanova, 2008; Titlyanov et al., 2008b) and indirect ones such as environmental modification and space preemption (Dobson, Hudson, 1986; Hudson, Greenman, 1998), production of allelochemicals (Bak, Borsboom, 1984; Paul, Puglisi, 2004; Gross, 2003; Titlyanov et al., 2007) and the stimulation of pathogenic bacteria (Nugues et al., 2004; Smith et al., 2006). Further, there have been reported cases of macroalgae directly killing and overgrowing neighbouring corals (McCook, 2001; Jompa, McCook, 2003; Nugues et al., 2004).

Not all algal species on coral reefs compete with corals for space (e.g., endophytic and epiphytic algae). Corals' main competitors for space include brown algae with large upgrowing thalli (the genera *Sargassum*, *Padina*, *Dictyota*, etc.), dense communities of algal turfs and toxin-producing algae. With the formation of algal communities on a damaged coral reef, the recovery of coral colonies and their expansion

over hard substrata may largely be impeded (Titlyanov, Titlyanova, 2008), depending on species-specific competitive abilities of algal thalli and coral colonies (Jompa, McCook, 2003; Birrell et al., 2005) and also on environmental conditions. However, even at this stage of coral/algal competition, adult coral colonies may still remain superior competitors in the absence of strong anthropogenic stresses.

It should be noted that algae can overgrow coral polyps only when they are damaged, weakened or under stressed conditions in general. In an experimental study involving algal species such as *Peyssonnelia conchicola*, *Corallophila kleiwegii*, *Centroceras clavulatum*, *Anotrichium tenue*, *Polysiphonia* spp. (Rh), *Lobophora variegata*, *Sphacelaria novae-hollandiae*, *S.tribuloides* (Ph), *Codium* spp., *Dictyosphaeria* spp. (Ch), these could overgrow colonies of *Porites lutea* only under the light intensity of less than 5% PAR<sub>0</sub> (Titlyanov et al., 2009a). This is related to the differences in light adaptation between algae and corals. It has been demonstrated that the majority of scleractinian coral species are associated with light levels of 1 - 80% PAR<sub>0</sub> (Titlyanov, Latypov, 1991), while red coralline algae can tolerate down to 0.1% PAR<sub>0</sub> (Littler, Littler, 1988).

Early life stages of many sessile species including hermatypic corals are typically competitively inferior to older and larger individuals (Sebens, 1989; Maida et al., 2001; Vermeij et al., 2009). In similar vein, large-sized, fleshy, coriaceous and foliaceous forms of marine plants as well as algal turfs can limit the success of smaller competitors (e.g., coral planulae, larvae of other sessile organisms, young coral colonies (Grant, 1977; Grizzle et al., 1996). It has been suggested that the effect of algae on corals is strongest during the coral's earliest benthic stages (Hughes, Jackson, 1985; Hughes, 1989, 1996; McCook et al., 2001; Vermeij, Sandin, 2008). Coral recruitment commonly declines when benthic algae become abundant in experimental (Rogers et al., 1984; Birrell et al., 2005; Hughes et al., 2007) and natural settings (Hughes, 1989; Birkeland, 1996; Edmunds, Carpenter, 2001; Vermeij, Sandin, 2008). Benthic algal assemblages have direct negative impacts on coral recruitment mainly through the preemption of settlement space (Birrell et al., 2005; Mumby et al., 2007; Vermeij et al., 2009). Additionally, algae can have indirect negative impacts on coral recruits through allelopathy (Gross, 2003; Kuffner et al., 2006).

Abundance of macroalgae is often attributed to their ability to actively overtake space previously occupied by corals, i.e. their presumed greater competitive capacity than corals, although there is little direct evidence supporting this hypothesis (McCook et al., 2001; Fong, Paul, 2011). Indeed, we argue that passive colonization of space previously occupied by stony corals that died from causes other than competition with algae (e.g., disease, bleaching) might often have been mistakenly interpreted as evidence of active competition between corals and algae (Vermeij et al., 2009). On the other hand, under certain circumstances macroalgae do seem to restrict the growth of coral colonies. In subtropical, high-latitude coral assemblages, polyps on the edges of acroporan (*Acropora*) colonies are often injured and killed by whipping movements of neighbouring macroalgae such as *Gelidium elegans*.

#### iv. Corals as superior competitors in coral reef recovery

Recovery rates of coral reefs can reach 10% per year, according to experimental ecophysiological studies conducted on healthy and damaged coral colonies (Titlyanov et al., 1998, 1999, 2000a, 2001a, b, c, d, 2005, 2006, 2007, 2008a, b, 2009a; Titlyanov, Titlyanova, 2002a, b, 2008, 2009, 2012a, b) and the monitoring of coral reefs after natural catastrophes in different regions of the world (Loch et al., 2002; Stobart et al., 2005; Guzman, Cortes, 2007; Wellington, Glynn, 2007; Baker et al., 2008; Rogers et al., 2008). During coral reef recovery, hermatypic corals are often superior competitors to macrophytes and cyanobacteria which do not impede coral recruitment. High competitive

abilities of hermatypic corals are related to various morphological and physiological aspects:

- (1) Long-term (tens to hundreds of years) presence and growth of certain coral colonies (Veron, 1986), while marine plants exist from days to a few years at most (Loban, Harrison, 1994). Corals are permanent (long-living) settlers on hard substrata and gradually overgrow other non-toxin producing organisms. At the same time, algae (toxic or large forms) constitute only a temporary impediment to corals as they may be replaced by other, non-toxic forms which cannot impede coral growth. This replacement may occur during seasonal changes in species composition of algal assemblages or under stressful conditions (disturbances by storms, grazing by animals, etc.). After disappearance of harmful algae, bleached polyps (which were under direct contact with toxic or large algae) could recover in 2 – 4 weeks and continue to occupy the substrate.
- (2) High regenerative capacity of corals. Newly-formed live tissues of corals can gradually overgrow the dead parts colonized by algae. Corals' ability to recover from damages, depending on the position, size, shape and type of the damage, has been well documented (Bak, Steward-van Es, 1980; Bak, 1983; Wahle, 1983; Rinkevich, Loya, 1989; Meesters et al., 1993, 1994; Meesters, Bak, 1995; Hall, 1997, 2001; Oren et al., 1997; Marshall, 2000). For example, the newly-formed tissues of the corals *Porites* spp. and *Montipora grisea* tightly adjoined to the substrate with the exception of the front line (Titlyanov et al., 2005). This portion of the live tissue edge (probably not calcified or slightly calcified) had no zooxanthellae and appeared as transparent stripes between live polyps and dead skeleton of the lesion. The width of the tissue stripe depended on coral species, e.g. 100 – 300 µm wide in *Porites lutea*, 50 – 100 µm in *P. cylindrica* and 50 – 200 µm in *P. rus*. The newly-formed coral tissue was able to go round impediments (Fig. 14) or rise above the impediments to overgrow. In the latter case, the impediment (live or lifeless object) was entombed into the coral skeleton.

A branched coral *Acropora* sp. and a foliaceous coral *Pavona divaricata* demonstrated different processes of lesion healing. After fast recovery (4 – 6 days) of damaged tissues, polyps began to occupy dead areas of the lesion by spreading and overgrowing with a thin blade-like formation consisting of soft tissue and hard skeleton. Sharp crystals (like spines or teeth), sometimes covered by live tissue (bearing zooxanthellae), and projected along the front line of the "blade". The blade did not adjoin firmly to the substratum and could rise above the lesion at a distance of 1 mm or more. As the blade expanded over

the substratum, its back portion fixed newly formed skeleton to the lesion, entombing all sessile organisms into the skeleton. Our research to date suggests that this mechanism of substrate colonization is characteristic of many encrusting coral species and widely observed on coral reefs.

Expansion of massive corals may be temporarily hampered by obstacles such as tall and dense algal turfs, large fleshy algal thalli or large sessile animals. In such cases, new polyps may form "bolsters" in front of and above the impediment (Titlyanov, Titlyanova, 2008) and these may eventually close up, entombing algae into the coral skeleton.

On damaged reefs, coral polyps coming into direct contact with algae tend to become bleached by allelopathic substances, abrasion, smothering or shading. However, bleached polyps may remain alive for a long time and recover after cessation of negative algal influences. For instance, experiments on the physical contact of *Porites lutea* colony fragments with a mat of the blue-green alga *Lyngbya bouillonii* demonstrated that the contact during one month inhibited growth and photosynthesis of the coral and bleached its polyps, due to a significant decline in zooxanthellae density and their total chlorophyll content (Titlyanov et al., 2007). These bleached fragments completely recovered in the absence of *L. bouillonii* under the light intensity of 30% PAR0 for two months. Similarly, our observations in Amakusa, Japan, showed that a colony of encrusting *Acanthastrea* sp. was observed to recover after having been covered by algae for several months and suffering from partial discoloration.

- (3) Advantage of size. In tropical and subtropical waters, healthy adult coral colonies of all growth forms except encrusting ones do not generally compete with algae because of their large sizes (height) surpassing algal assemblages, conceding only to Sargassaceae species on rare occasions. Coral colonies shade the bottom space immediately underneath and deprive fast-growing algae of necessary light. On shaded substrates under coral colonies, mainly slow-growing coralline algae settle, which in turn promote the attachment of hermatypic corals' planulae (Fong, Paul, 2011).
- (4) Different ways of feeding in corals, including photosynthesis of zooxanthellae, predation, consumption of particles of organic (animal) origin and digestion of own zooxanthellae (Titlyanov et al., 1996; Titlyanov, Titlyanova, 2002a). These allow corals to survive under unfavorable conditions such as low light conditions and waters poor in nutrients. For instance, the branched coral *Stylophora pistillata* was shown to survive and acclimate to a wide range of light intensities from 0.8 to 95% PAR0. Acclimation to low light conditions (8 and 30%

PAR0) involved maximizing the light harvesting capacity by increasing photosynthetic pigment concentration in zooxanthellae and zooxanthellae population density in coral branches. Under the extremely low light level (0.8% PAR0), the coral lost zooxanthellae by digestion and retained zooxanthellae-accumulated high concentrations of chlorophyll. The photoacclimation process is dynamic and immediate. Changes in pigment concentration in zooxanthellae occurred within 2 - 4 days and changes in zooxanthellae population density within 40 days. Zooxanthellae population densities were regulated by changes in the rates of division and degradation (digestion) of symbiotic cells (Titlyanov et al., 2001c).

Predation may be interpreted as adaptation to low light levels. Under the illumination of 2% of PAR0, capture and ingestion of *Artemia salina* nauplii by the coral *S. pistillata* were stimulated, with increasing ratios of ingested to killed nauplii; i.e., predation became more efficient than under 20 or 90% PAR0. It has been mentioned that under high/moderate light, corals most actively hunt in early morning hours, whereas under conditions of shading, they hunt throughout the day. When light is deficient, predation appears to be the major source of obtaining food in corals (Titlyanov et al., 2000 b, c). A decrease in photosynthesis of *S. pistillata* under light limitation also induced an increase in both chlorophyll concentration and zooxanthellae population density. Both responses require nitrogen that is generally insufficient in seawaters above coral reefs. For maintaining these responses the coral is capable of using nitrogenous compounds derived from captured zooplankton prey (Titlyanov et al., 2000c).

- (5) Corals' capacity to clean off organic and inorganic sediments from colony surfaces. Corals exhibit both active and passive removal of sediment particles (Lasker, 1980). They have a variety of mechanisms for coping with sediments including the use of their tentacles and cilia, stomodeal distension through water uptake, and entanglement of particles in mucus which later sloughs off the colony surface (Hubbard, Pocock, 1972). Where currents are strong, water movement will help keep sediment particles from settling on colony surfaces, and corals will have to spend less energy in sediment rejection. Species differ in their ability to reject sediments, colony and polyp morphology playing an important role (Hubbard, Pocock, 1972). The amount and type of sediment will influence the ability of a coral to maintain its surfaces free of sediments.

Colonies of some genera of hermatypic corals exhibit changes in orientation and morphology, which appear to occur in response to sediment stress (Bak, Elgershuizen, 1976). This would reduce the possibility of

settlement of spores and planulae on colony surfaces and help corals survive under conditions of high sedimentation. Macroalgae under such conditions become covered with sediments that limit their production and absorption of nutrients (Titlyanov et al., 2011a, b). Only crustose calcareous algae have a mechanism of surface cells' sloughing (Keats et al., 1994).

(6) The ability of coral planulae to attach to and grow on the thalli of competitors. Calcareous algae are considered the preferred substrate for attachment and development of coral planulae. When planulae receive certain chemical triggers secreted by crustose coralline algae, they stop swimming, attach to the substrate and develop into the primary polyps (Harrison, Wallace, 1990; Harrington et al., 2004; Golbuu, Richmond, 2007). It was shown that this induction effect of settlement is species-specific (Heyward, Negri, 1999; Golbuu, Richmond, 2007). Microbes living on algae may also induce/stimulate planula settlement and metamorphosis (Neumann, 1979; Richmond, 1987; Morse et al., 1988; Zaslav, Benayahu, 1996; Heyward, Negri, 1999). Moreover, settlement and survival of coral planulae may be influenced by independent as well as synergistic effects of macroalgae and microbes (Vermeij et al., 2009).

These suggest that not only red calcareous algae but also other representatives of algal turfs may serve as substrata for planula settlement. When 11 macroalgal species were tested on their effects on the swimming and settlement of *Platygyra daedalea* larvae, algal turfs and crustose calcareous algal groups had minor effects on coral settlement, while upright calcareous and fleshy macroalgae inhibited settlement (Diaz-Pulido et al., 2010).

(7) Coral fragments' capacity to attach to and grow on hard substrata and also the possibility of their non-attached existence. This feature is considered of importance in coral reef restoration after strong typhoon/tsunami damages. Coral fragments or even colonies could be dislodged, transported by water movements and relocated in new habitats. Under certain conditions, coral fragments may successfully attach to hard substrata or grow together with live colonies. Unattached fragments may survive for a long time on soft substrates, often acquiring a spherical form due to rolling (Veron, 1986; our own observations). In contrast, algae detached from the substrate are most likely to perish.

(8) Mobility of corals. Some coral species, most notably of the Family Fungiidae, are capable of actively moving over hard and soft substrata (Veron, 1986), which allows them to aggregate in favorable microhabitats where competition with algae may be reduced.

Thus, hermatypic corals tend to be competitively superior to macroalgae under natural/semi-natural conditions of reef ecosystems. While the competitive capacity of some macroalgae is enhanced under the advanced states of seawater contamination (Rosenberg, 1985; Doering et al., 1995; Harlin, 1995; Fletcher, 1996; Raffaelli et al., 1998; Taylor et al., 1999; Thornber et al., 2008; Nixon, Buckley, 2002), the combination of traits described here gives credence to the view that in the majority of cases corals outcompete algae on coral reefs damaged by natural catastrophes.

#### IV. CONCLUSION

In conclusion, we draw attention to the positive roles of algae in first colonizing newly-formed substrata after disturbance events and in contributing to the restoration of coral-dominant states of undamaged reefs. It may be argued that damaged coral reef ecosystems can regain homeostasis that has been lost during a natural catastrophe. We suggest that this is mainly achieved through the colonization of newly formed substrates by marine algae, with the following characteristics:

- (1) Maintenance of high ecosystem productivity through settlement of highly productive morpho-functional algal forms such as fine filamentous, filamentous and lamellar green and red algae (Sergeeva et al., 2007). While in healthy coral reefs symbiotic microalgae-zooxanthellae are the main primary producers, multi-cellular algae and cyanobacteria may often become the main primary producers that occupy denuded substrata after a catastrophe. Photosynthetic rates (per unit area of substrate) of algal communities may be equal to or greater than that of zooxanthellae in hermatypic corals (Littler, Littler, 1988; Littler et al., 1991; Titlyanov et al., 2007). Algal communities temporarily become the main supplier of organic matter and energy in a damaged reef ecosystem.
- (2) Protection of coral reef basis and newly formed carbonate substrata (dead coral colonies) from erosion and continuation of carbonate reef base building. Calcareous algae (Littler, Littler, 1988) help cement dead colonies and their debris into the carbonate reef base.
- (3) Colonization of Vacant substrates by algae enhances the biodiversity of an entire reef assemblage (Sergeeva et al., 2007; Baker et al., 2008; Fong, Paul, 2011).
- (4) Symbiotic relations between algae and corals also promote homeostasis and coral reef recovery in damaged reef systems through transport of assimilates from endolithic symbiotic algae (e. g., *Ostreobium quekettii*) to coral tissue (Fine, Loya, 2002; Titlyanov et al., 2008b, 2009b), which

- intensifies during a bleaching episode (Fine, Loya, 2002), or by coral digestion of own zooxanthellae that intensifies under extreme conditions (low light, starving, osmotic shock) (Titlyanov et al., 1996).
- (5) Release of secondary chemicals by encrusting calcareous algae (or their bacterial biofilm) promoting planula settlement and growth on their surfaces (Hadfield, Paul, 2001; Negri et al., 2001; Vermeij et al., 2009; Ritson-Williams et al., 2010).
- (6) Planulae and young colonies attached to calcareous algae at the base of algal turf are protected from predatory/grazing organisms and from desiccation and bleaching in the intertidal. Coral growth is enhanced by the accumulation of zooplankton and other organisms in algal turfs (Sorokin, 1990).
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## Helminth Parasites of Two Freshwater Fishes (*Oreochromis niloticus* and *Clarias gariepinus*) in Jibia Earth Dam, Katsina State, Nigeria

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**Abstract-** Investigation on helminth parasitic fauna of two freshwater fishes *Oreochromis niloticus* and *Clarias gariepinus* was carried out from August 2016 to January 2017. A total of 242 fish samples comprising of 117 *Oreochromis niloticus* and 125 *Clarias gariepinus* of different weights and length groups were collected from Jibia earth dam, Katsina state, Nigeria and subjected to parasitological examination. The overall prevalence of infection recorded in the two fish species was 38.43%. *Clarias gariepinus* recorded the highest prevalence of infection of 46.40%. Helminth parasites recovered were mainly the trematode: Neascussp, nematodes: *Procamallanus laevionchus* and *Contraecaecum* sp, cestodes: *Polygonchobothrium clarias*, *Bothriocephalus aegyptiacus* and *Proteocephalus glanduliger* and acanthocephalan: *Neoechinorhynchus rutili*. Helminthic infections were recorded in the skin, stomach and intestine though majority of infection was found in the intestine.

**Keywords:** *jibia, katsina, helminth parasites, oreochromis niloticus and clarias gariepinus.*

**GJSFR-C Classification:** FOR Code: 060204



HELMINTHPARASITESTWOFFRESHWATERFISHESOREOCHROMISNILOTICUSANDCLARIASGARIEPINUSINJIBIAEARTHDAMDAMKATINASTATENIGERIA

Strictly as per the compliance and regulations of:



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# Helminth Parasites of Two Freshwater Fishes (*Oreochromis niloticus* and *Clarias gariepinus*) in Jibia Earth Dam, Katsina State, Nigeria

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**Abstract-** Investigation on helminth parasitic fauna of two freshwater fishes *Oreochromis niloticus* and *Clarias gariepinus* was carried out from August 2016 to January 2017. A total of 242 fish samples comprising of 117 *Oreochromis niloticus* and 125 *Clarias gariepinus* of different weights and length groups were collected from Jibia earth dam, Katsina state, Nigeria and subjected to parasitological examination. The overall prevalence of infection recorded in the two fish species was 38.43%. *Clarias gariepinus* recorded the highest prevalence of infection of 46.40%. Helminth parasites recovered were mainly the trematode: Neascus sp, nematodes: *Procamallanus laevionchus* and *Contraecaecum* sp, cestodes: *Polyonchobothrium clarias*, *Bothriocephalus aegyptiacus* and *Proteocephalus glanduliger* and acanthocephalan: *Neoechinorhynchus rutili*. Helminthic infections were recorded in the skin, stomach and intestine though majority of infection was found in the intestine. The result showed that the prevalence of infection increased with increase in the hosts size (length and weight), and infection was independent of fish sex. Infection of the helminth parasites was more prevalent in the dry season than the rainy season. No significant difference in the percentage of parasitic infection in relation to the size, sexes and season ( $p > 0.05$ ) was recorded; however, significant difference ( $p < 0.05$ ) exist in infection rate between the two species examined. Most of the parasites were recovered from the intestine, with a few from skin and stomach, therefore, removal of these fish parts and thorough cooking of fish before consumption will ensure human safety.

**Keywords:** jibia, katsina, helminth parasites, oreochromis niloticus and clarias gariepinus.

## I. INTRODUCTION

Due to higher biological value of fish (high protein retention, assimilation, low cholesterol content, and safety), it has continued to be important in the diet of humans in tropical Africa and different parts of the world (Akinsanya, 2015). Fish accounts for more than 40% of the protein diet of two-thirds of the global population (Bichi and Yelwa 2010). As population has grown, incomes have increased and nutritional benefits of fish have become better known, demand for fish has increased. In recent times, there has been a tremendous increase in the development of fish farming and culture due to increase need for animal protein. In Nigeria, there is an estimated 12.5mha of freshwater surface area of

lakes, reservoirs and ponds which are capable of producing 521,000 metric tons of fish but these have not succeeded in attaining fish food sufficiency (Biu et al., 2014).

Parasite is an important group of pathogen causes infection and diseases of fish both in freshwater and marine environments (Chandra, 2006). Parasitic infection causes production and economic losses through direct fish mortality, reduction in fish growth, fecundity and increase in the susceptibility of fish to diseases (Salawuet et al., 2013). Parasitic infestations are therefore becoming threats for fish health management and aquatic crop production (Chandra, 2006).

With the increasing interest in aquaculture, a considerable amount of information is available on helminth fauna of freshwater fishes. In Nigeria, most studies on fish parasites have been carried out in southern part; however, literature from the northern part is scanty. The present study was therefore undertaken to investigate the helminth parasites from two freshwater fish species, *Oreochromis niloticus* and *Clarias gariepinus* inhabiting Jibia earth dam of Katsina state, Nigeria.

## II. MATERIALS AND METHODS

### a) Study Area

Jibia dam is located in Jibia local government area of Katsina state. Jibia lies between latitude 13° 05' N and 7° 13' E and longitude 13° 09' N and 7° 23' E. It has a total human population of 169,748 and total land mass of about 1,037km<sup>2</sup>. The dam lies on the coordinates 13° 04' 18' N and 07° 15' 06' E. It has a height of 23.5m, a length of 3,660m and a total capacity of 142million m<sup>3</sup> (Figure 1).

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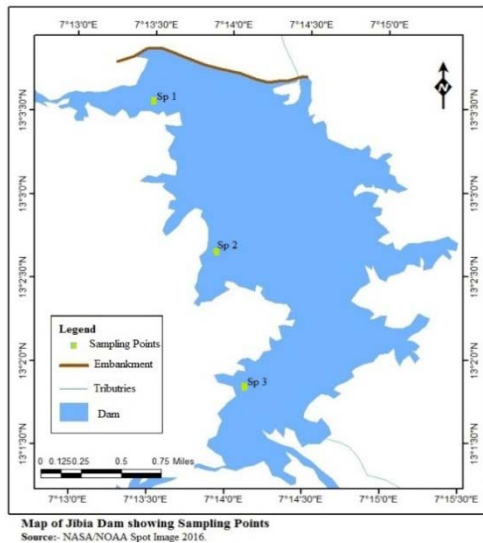


Figure 1: Map of Jibia Dam showing the sampling Points

b) Samples Collection

From August 2016 to January 2017, a total of 242 randomly selected fishes comprising of 117 *Oreochromis niloticus* and 125 *Clarias gariepinus* of different weights and length groups were collected from three different sampling points with the assistant of local fishermen and examined. Sample collections were done in the morning between 06:00 to 08:00am. Water from the dam was added to the samples before being transported to the Biological science laboratory of Umaru Musa Yar'adua University Katsina in aerated plastic containers.

c) Morphometric Study

At the laboratory, the fishes were given serial number and then fish morphometric (measuring of weight and length) prior to dissection was done. Using transparent ruler, the total length of each fish was taken from the tip of the snout (with the mouth closed) to the extended tip of the caudal fin while the standard length was obtained by subtracting the length of the caudal fin from the total length and recorded to the nearest 0.5 centimetre (cm). After draining excess water, the weight (w) of the same fish was obtained to the nearest 0.1g using a weighing balance (Scout Pro SPU202). Sex determination of the fish species was done by visual examination of the anal opening for the presence of papilla just before the anal fin is indicative for a male species while the absence of the papilla indicates a female species. This was consequently confirmed by the presence or absence of testis or ovaries during dissection (Akinsanya, 2015).

d) Examination of fish for parasites

As examination progresses, dead fishes were removed and examined immediately while the live ones were kept in a plastic aquaria containing water from the dam, and examined subsequently. Lived fishes were

killed by cervical dislocation to ease examination (Ajala and Fawole, 2014).

e) Ectoparasites

The entire external body surfaces of a freshly caught fish was thoroughly examined for ecto-parasites using hand lens. Mucous scrapings from dorsal part of the body of fish, lateral and tail ends were placed on clean glass slide, with a drop of saline added and were examined under x10 and x40 objective lenses of compound microscope. A small section of the affected body surfaces were cut and placed in aqueous formalin for 30 minutes. The mixture was shaken vigorously to dislodge relaxed helminthes. The operculum of fish was cut open with scissors and gills were exposed. Gill arches and gill filaments were placed in different Petri dishes containing normal saline and were observed with hand lens, dissecting and compound microscope for parasites (Biu et al., 2014; Okoyeet al., 2014).

f) Endoparasites

Fish samples were placed dorso-ventrally on dissecting board and fixed to prevent movement. The body cavity was opened with the aid of scissors and the mesentery and connective tissues, connecting loops of the gut and the liver were cut and the organs separated. The gut was then stretched out, placed in a large Petri dish and cut into four regions (oesophagus, stomach, intestine and duodenum). Each section was then placed in a separate labeled dish. The separated gut sections were opened by longitudinal incision to expose the inner surface which was washed with very little quantity of distilled water into labelled test tubes. A drop of the residue was placed on the slide, and observed under x10 and x40 objectives of dissecting microscope for the various parasites. This was repeated until the entire residue was examined (Bichi and Yelwa 2010; Ajala and Fawole, 2014).

g) Isolation and Identification of Helminth Parasites

Most of the parasites were recognized by their wriggling movement on emergence from their host. Parasites were picked with Pasteur pipette and forceps. Parasites obtained were counted, labeled with the serial number of the fish and placed in physiological saline overnight to allow them stretch and relax; they were then fixed and stained using acetocarmine and lactophenol. Identification of the isolated parasites to species level was done by comparing observed parasites using keys provided by Yamaguti, (1959 and 1961), Gibson, (1996) and Barson and Avenant-Oldewage, (2006)

h) Statistical Analysis

The relationships between factors such as length, weight, sex, species and season were obtained using Analysis of Variance (ANOVA). All statistical analysis were done using Graph Pad InStat Software, 2016. Values equal to or less than 0.05 ( $p \leq 0.05$ ) were regarded as significant.

### III. RESULT

Of 242 samples of both *O. niloticus* and *C. gariepinus* examined in the study area, 93(38.43%) were affected by different helminth parasites. Out of 125 *C. gariepinus* examined, 58(46.4%) were affected by the parasites. However, of the 117 *O. niloticus*, 35(29.91%) were affected by the helminth parasites and infection was found to be statistically significant ( $p < 0.05$ ) between the species examined. Worm burden was high in *C. gariepinus* with one hundred and fifty (150) helminth parasites isolated than *O. niloticus* with ninety one (91) isolated parasites in both single and mixed infections (Table 1). During the present study, three species of cestodes (*P. clarias*, *B. aegyptiacus* and *P. glanduliger*), two species of nematodes (*P. laevionchus* and *Contracaecum* sp) and one specie each of trematode (*Neascussp*) and acanthocephalan (*N. rutuli*) have been isolated. The parasites infected two fish organs (Intestine and stomach). *P. clarias*, *B. aegyptiacus* and *P. glanduliger* were found in the in the intestine of

*C. gariepinus*. Larval stages of *Contracaecum* sp and *N. rutuli* was found in the intestine of the both fish species whilst of *P. laevionchus* occurred either in the intestine or stomach region of *C. gariepinus* and only in the intestine of *O. niloticus*. (Table 2). Table 3 shows the prevalence of helminths in relation to sex of the two fish species. In this study, prevalence of infection was found to be independent of fish sexes and the differences obtained were not statistically significant ( $p > 0.05$ ). Infection was high in dry season than the rainy season in both fish species, but no seasonal variation in prevalence was observed between the two seasons ( $p > 0.05$ ) (Table 4). Relationship between host size (length and weight) and percentage of infection are shown in Table 5 and 6. Fish between the total length group of 22-25cm in *O. niloticus* and 35-39cm in *C. gariepinus* were more parasitized than fish of other length class (Tables 5). Infection was more pronounced in fish of the weight class 161-180g in both fish species examined (table 6), although association of host size and infections were not statistically significant in this study.

Table 1: Overall Percentage of Infection in the study areas

Host	No. Examined	No. Infected	Prev. (%)	Total No. of Parasites recovered	Intensity
<i>O. niloticus</i>	117	35	29.91	91	2.6
<i>C. gariepinus</i>	125	58	46.40	150	2.5
Total	242	93	38.43	241	2.59

Prev. = Prevalence

Table 2: Parasites Distribution in organs of *O. niloticus* and *C. gariepinus* in the study areas

Parasites	<i>O. niloticus</i> (N=117)		<i>C. gariepinus</i> (N=125)		
	Skin/fin	Intestine	Skin/fin	Intestine	Stomach
Trematode					
<i>Neascussp</i>	09(7.69%)		09(7.2%)	-	-
Cestode					
<i>B. aegyptiacus</i>		-	-	08(6.4%)	
<i>P. clarias</i>	-	-	-	11(8.8%)	-
<i>P. glanduliger</i>		-	-	05(9.6%)	-
Nematode					
<i>P. laevionchus</i>	-	17(14.53%)	-	19(15.2%)	05(4.0%)
<i>Contracaecum</i> sp	-	15(12.82%)	-	14(11.2%)	-
Acanthocephalan					
<i>N. rutuli</i>	-	07(5.98%)	-	06(4.8%)	-
Total	09(7.69%)	39(33.33%)	09(7.2%)	63(50.4%)	5(4.0%)



Table 3: Sex and infection of Helminth parasites in the study area

Sex	<i>O. niloticus</i>			Host	<i>C. gariepinus</i>		
	No. Examined	No. Infected	Prev. (%)	No. Examined	No. Infected	Prev. (%)	
Male	51	19	37.35	73	33	45.21	
Female	66	16	24.24	52	25	48.08	

Prev. = Prevalence

Table 4: Seasonal occurrence of Helminth parasites in the study area

Season	<i>O. niloticus</i>			Host	<i>C. gariepinus</i>		
	No. Examined	No. Infected	Prev. (%)	No. Examined	No. Infected (%)	Prev.	
Rainy	71	16	22.54	60	22	36.67	
Dry	46	19	41.30	65	36	55.38	

Prev. = Prevalence

Table 5: Relationship between total length and percentage of infection in *O. niloticus* and *C. gariepinus* in the study area

Length (cm)	<i>O. niloticus</i>			Host	<i>C. gariepinus</i>		
	No. Examined	No. Infected	Prev. (%)	No. Examined	No. Infected (%)	Prev.	
10-13	05	-	-	-	-	-	
14-17	56	15	25.	-	-	-	
18-21	39	12	30.77	36	13	36.11	
22-25	17	08	47.06	47	21	44.68	
26-29	-	-	-	26	14	53.85	
30-33	-	-	-	09	05	55.55	
35-39	-	-	-	07	05	71.43	

Prev. = Prevalence

Table 6: Relationship between weight and percentage of infection in *O. niloticus* and *C. gariepinus* in the study area

Weight (g)	<i>O. niloticus</i>			Host	<i>C. gariepinus</i>		
	No. Examined	No. Infected	Prev. (%)	No. Examined	No. Infected	Prev. (%)	
41-60	13	02	15.38	22	08	36.36	
61-80	24	05	20.83	35	15	42.86	
81-100	39	11	28.21	26	12	46.15	
101-120	29	09	31.03	17	08	47.06	
121-140	10	07	70.0	19	11	57.89	
141-160	01	-	-	04	02	50.0	
161-180	01	01	100	02	02	100	

Prev. = Prevalence

#### IV. DISCUSSION

The overall prevalence of 38.43% observed in the present study was low particularly when compared to the 60.23% reported by Olofintoye (2006), 59.2% reported by Oyedinekeet *al.*, (2010) and 56.4% reported by Amaechi, (2014). It was however high when compared with the 18.70% reported by Biu and Nkechi (2013) and 18.5% by Ogbeibuet *al.*, 2014. It is worthy to

note that infection rates vary from one region to another and that a number of factors like endemicity, availability of intermediate host, susceptibility of a definitive host, amongst others, determine to a large extent the rate of infection (Biu and Akorede, 2013). *C. gariepinus* had the highest prevalence of 46.40%. The highest prevalence of parasites in *C. gariepinus* may be due to several factors which include feeding habit and diet of fish, habitat, immuno - competence of the fish, as well as the

behavioral pattern of the fish (Eyo *et al.*, 2014). Different helminth parasites belonging to different groups namely; two nematodes (*P. laevionchus* and) three cestodes (*P. clarias*, *B.aegyptiacus* and *P. glanduliger*) and one each of one trematode (*Neascus* sp) and acanthocephalan (*N. rutuli*) were observed in this study. The occurrence of these parasites in the study area was not surprising as they have been reported previously from the same species or related species elsewhere (Uruku and Adikwu, 2017). Majority of the parasites recovered were found in the intestine with very few in the stomach and skin/fin. The high prevalence recorded in the intestine in this study cannot be unconnected with the findings of Dan-kishiya *et al.*, (2013) who reported higher number of parasites in the intestine than the stomach and attributed it to several factors among which, was the presence of digested food or due to the greater surface area presented by the intestine. Similarly, Bichi and Yelwa, (2010) also reported high prevalence of helminth parasites in the intestine than the stomach and argued that regional localization in the gut can be attributed to several factors, such as Hydrogen ion concentration, chemotactic response as well as food reserve. Nematodes were recovered from both the stomach and intestine, whereas the cestode and acanthocephalan showed preference for the intestine. This could be due to the fact that nematodes have relatively developed alimentary canal and could easily move around any area of the host alimentary canal to feed on digested and semi-digested food (Kawe *et al.*, 2016).

In this study, infection was found to be independent of fish sexes. The high prevalence of parasitic infestation in males than the females *O. niloticus* in this study agrees with the reported work of Anosike *et al.*, (1992) and Oniye *et al.*, (2004) who reported high prevalence of infection in male fish than the female. The highest prevalence of male than female fishes observed in this study may be as a result of difference in reproductive investment by male and female fish, immuno-suppression by steroid hormone during spawning, competition for mate and cost of territorial defense (Eyo *et al.*, 2014). Contrary to the aforementioned, the higher prevalence of infection obtained among female fishes of *C. gariepinus* agrees with the findings of Emere and Egbe (2006), Ayanda (2009) and Omeji *et al* (2011), who reported higher parasitic infection in female fishes and attributed it to the physiological state of the females, as most gravid females could have had reduced resistance to infection by parasites. In addition, their increased rate of food intake to meet their food requirements for the development of their egg might have exposed them to more contact with the parasites, which subsequently increased their chance of being infected. Variations obtained in parasitic infection among the sexes of fish studied were not significant ( $P>0.05$ ) implying that

higher infection rates in either the male or female were simply by chance (Biu and Akorede, 2013). The high prevalence of infection obtained in dry season in this study agrees with the report of Fawole and Akinsanya, (2010), but disagrees with the findings of Bichi and Bizi (2002). Seasonal variation in the occurrence of these parasites may be attributed to reduce in water volume, resulting in much contact between the parasites and fish, thus leading to a relatively higher prevalence in the dry season (Uruku and Adikwu, 2017). In relation to size (weight and length) it was observed in this study that the percentage infection increased with increasing size. Similar observations were reported by Ayanda (2009), Olurin and Samorin, (2006), Mohammed *et al.*, 2009, and Oniye *et al.*, 2004 that the longer and heavier the fish was, the greater the susceptibility to parasitic infection. This could be due to the fact that bigger fish cover wider areas in search of food than the smaller ones and as a result, they take in more food and this could expose them more to infestation by parasites.

## V. CONCLUSION

In conclusion, the report would not have succeeded in identifying all the parasites that may likely be found on the studied fishes. It is therefore, recommended that follow up surveys on the lifecycle of the major parasites should be done at certain intervals in order to identify any change in the trends of possible fish parasites that could affect the fish populations. Some of the helminthes isolated are of zoonotic potential, thus, removal of the intestine and thorough cooking of fish will ensure humans safety even when they consume infected fish.

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## First Report of Family Physidae (Gastropoda) with *Physa acuta* as its Representative from Freshwaters of Chandigarh (U.T.), India

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**Abstract-** Physidae is the most world widely dispersed and commonly encountered family of freshwater pulmonates. *Physa acuta* Draparnaud, 1805, one of its common, occurring species belongs to Family Physidae of Class Gastropoda. It is a globally invasive freshwater gastropod species that had been recorded from only some states (10) of India till date. The present study reports the occurrence of this family along with the species for the first time from the freshwater bodies of U.T. Chandigarh. The species identification has been made by external morphology and anatomical characteristics like penial complex. The family has shown its dominance in still waters and slow-moving water bodies, rich in organic matter while the species has been found in other freshwater aquatic ecosystems too, though in very less percentage.

**Keywords:** *physidae, physaacuta, pulmonate, gastropod, penial complex, preputial gland, preputium.*

**GJSFR-C Classification:** FOR Code: 060204



Strictly as per the compliance and regulations of:



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Maansi <sup>α</sup> & Meenu Wats <sup>σ</sup>

**Abstract-** Physidae is the most world widely dispersed and commonly encountered family of freshwater pulmonates. *Physa acuta* Draparnaud, 1805, one of its common, occurring species belongs to Family Physidae of Class Gastropoda. It is a globally invasive freshwater gastropod species that had been recorded from only some states (10) of India till date. The present study reports the occurrence of this family along with the species for the first time from the freshwater bodies of U.T. Chandigarh. The species identification has been made by external morphology and anatomical characteristics like penial complex. The family has shown its dominance in still waters and slow-moving water bodies, rich in organic matter while the species has been found in other freshwater aquatic ecosystems too, though in very less percentage.

**Keywords:** *physidae*, *physaacuta*, *pulmonate*, *gastropod*, *penial complex*, *preputial gland*, *preputium*.

## I. INTRODUCTION

Physidae is the most world widely dispersed and commonly encountered family of freshwater pulmonates with about 80 species belonging to 23 genera. (Taylor 2003). The shells of the family are sinistral (left-handed or anticlockwise spirally coiled), distinguished by lack of an operculum, showing higher spiral shape and the animal showing a pair of sensory tentacles with eyes at their base.

*P. acuta*, commonly referred to as acute bladder snail or tadpole snail or sewage snails or pouch snails, is an alien and invasive freshwater pulmonate gastropod (Chilka Saha *et al.*, 2016). It is a North American species introduced into Europe from where it reached Africa (Brown 1980, Charles L. *et al.*, 2016) and South East Asia (Appleton 2003). This North American sewage snail has spread globally as an invasive species throughout the continents of Asia (Ali 1993), Africa (Brackenbury and Appleton, 1993; Appelton and Miranda, 2015), Australia (Zukowski and Walker, 2009), Europe (Semenchenko *et al.*, 2008; Raković *et al.*, 2016) and South America (Núñez 2010).

The first record of the occurrence of *P. acuta* in India (Pune, Maharashtra) has been reported by Subbha Rao in 1994). Later on this species had also been reported from varied freshwater bodies of other states of India by different scientists viz, Calcutta (Raut *et al.*,

1995; Soujitaet *et al.*, 2016); Delhi (Sury Rao *et al.*, 1997); Assam (Devi *et al.*, 2006); Jammu and Kashmir (Poonam *et al.*, 2013); Andhra Pradesh (M. Karuthapandi 2013); Himachal Pradesh (Tulika Biswas 2015); Uttarakhand (Pemola Devi 2015); West Bengal (ChilkaSaha 2016); Madhya Pradesh (Rita Bhandari 2016) and Haryana (Wats *et al.*, 2017). Due to its invasive nature, it is instrumented as a model organism to study interspecific competitions (Brown 1982), life history evolution (Crowl&Covich 1990) and population genetics (Dhillon & Wethington 1995). This species also act as bio-indicator of water quality as it is tolerant to pollution and brackish waters (Karuthapandiet *et al.*, 2013).

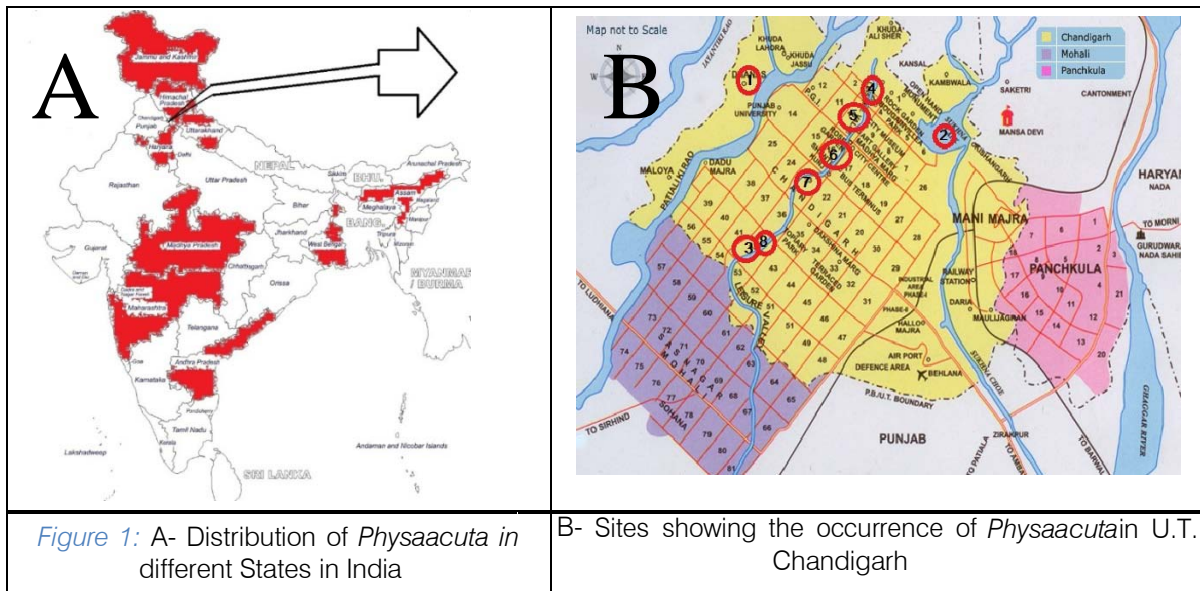
*P. acuta*, the freshwater snail, feeds upon algae, diatoms, detritus, other organic wastes etc. It is an air-breathing and hermaphrodite animal. Shells are egg-shaped with a pointed tip. The color may vary from light-yellow to brown. The shells of this species have a long and widely open aperture and are non-operculated. Externally, shell looks thin and corneous exhibiting some level of transparency. Indian literature reveals that *P. acuta* has been commonly found to be an inhabitant of ponds, streams, river, rice fields and municipality drains. (Strong *et al.*, 2008; Chilka Saha 2016) In the present study, the existence of *P. acuta* has been noted from varied types of freshwater bodies. The species is found to exist in lotic and lentic habitats, comparatively cleaner, moderately as well as heavily polluted water bodies, shallow as well as in deep waters. The current work is the first-time report of the occurrence of the family Physidae from the freshwaters in U.T. Chandigarh with *P. acuta* as its representative.

## II. METHODOLOGY

### a) Study Area

The study was carried out in the City Beautiful, Chandigarh, U.T., India (76°47'14" E and 30°44'14" N) located in the foothills of the Shivalik range. Chandigarh is famous for its three artificial lakes having three freshwater rivulets also, with one passing through its heart (N-choe) and other two in its vicinity (Sukhna-choe and Patiala Ki Rao). The current study was carried out at eight sites include one site, each from three lakes and five locations in one of the rainwater-fed rivulet (N-choe) passing through the center of the city. (Fig. 1B) The study was carried out for a year (May 2016-2017).

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i. Site Location

Following sites had been scanned for the presence of the given family and its representative.

1. Dhanas Lake (Dhanas Village, near sector-38, Chandigarh)
2. Sukhna Lake (Sector-1, Chandigarh)
3. New Lake (Sector-42, Near DR. Ambedkar Institute of Hotel Management, Chandigarh)
4. N-Choe (Bouganvillia Garden, Sector-4, Chandigarh)
5. N-Choe (Leisure Valley Garden, Sector-10, Chandigarh)
6. N-Choe (Rose Garden, Sector-16, Chandigarh)
7. N-Choe (Children Traffic Park, Sector-23, Chandigarh)
8. N-Choe (Behind Govt. Girls College, Sector-42, Chandigarh)

b) Sample Collection

The soil was sampled from littoral and benthic zone of water bodies manually as well as with the help of modified Dandy sampler (Fig. 2 C). Some mollusks from the littoral zone were picked manually. The collected samples were packed in polythene bags and were taken to the laboratory for segregation and identification of molluscan fauna. In the laboratory, soil samples were sieved using Standard Test Sieves (as per IS:460) of different mesh sizes (BSS 75mm, 44mm, 30mm, 10mm, and 4mm) (Fig. 2 A, B). Shells were collected manually from sieves (75mm, 44mm and 30mm) for cleaning and identification. The family identification was done by following the keys given by Ramakrishna and Dey 2007 and the species identification was done according to the anatomical features mentioned by Taylor, 2003. Morphometry of the shells was carried out for different (7) parameters like:

1. Height of shell (H)
2. Width of shell (B)
3. Height of spire (Hs)
4. Height of body whorl (HBw)
5. Height of aperture (Ha)
6. Width of Aperture (Ba)
7. Height of Penultimate (Hp)



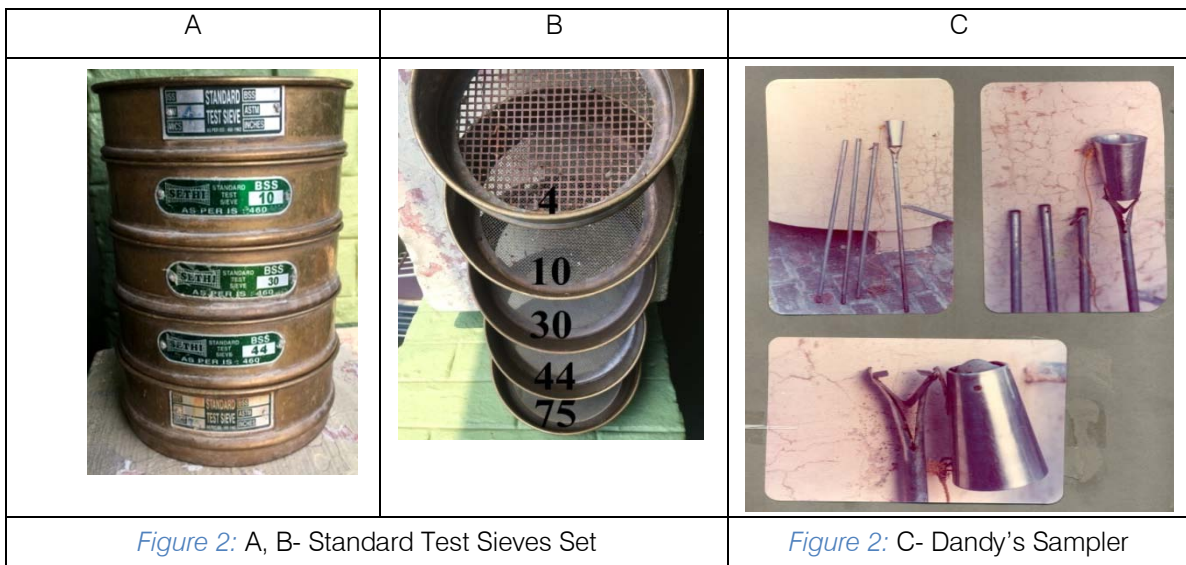


Figure 2: A, B- Standard Test Sieves Set

Figure 2: C- Dandy's Sampler

Shells were examined for their external morphology. The soft parts of the gastropod were taken out by keeping the live animals in warm water. On the protrusion of head and body parts, a few drops of the saturated solution of menthol were poured into the water to narcotize the animal. After that the soft parts of the animal were taken out by inserting a fine needle in its foot. The animal was dissected to take out its penial complex to identify and confirm the species of *Physa*.

### III. OBSERVATIONS AND RESULTS

*P. acuta*, the only representative of family Physidae in the current study, had already been reported from 10 states of India (Fig. 1A). The present investigation, attempts to claim the first ever study to show the inhabitation of the family and its representative, *P. acuta* from different freshwater ecosystems in U.T., Chandigarh.

#### a) Taxonomic Status

Table

<ul style="list-style-type: none"> <li>Kingdom: Animalia</li> <li>Subkingdom: Bilateria</li> <li>Infrakingdom: Protostomia</li> <li>Superphylum: Lophozoa</li> <li>Phylum: Mollusc</li> <li>Class: Gastropoda</li> </ul>	<ul style="list-style-type: none"> <li>Sub class: Pulmonata</li> <li>Super family: Physoidea</li> <li>Order: Bassomatophora</li> <li>Family: Physidae</li> <li>Genus: <i>Physa</i></li> <li>Species: <i>acuta</i></li> </ul>
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#### b) External morphology of the shell (*P. acuta*)

Shell is sinistral, elongate-ovate or egg-shaped, smooth, moderately lustrous and translucent. There are close-set lines of growth, spire short with slightly impressed sutures and whorls are regularly and rapidly decreasing in size with a pointed top. The aperture is large about  $\frac{3}{4}$ <sup>th</sup> of the total length of the shell; operculum is absent. The outer lip is thin and slightly deflected out, the umbilicus closed, parietal callus and columellar fold well marked. (Fig. 3)

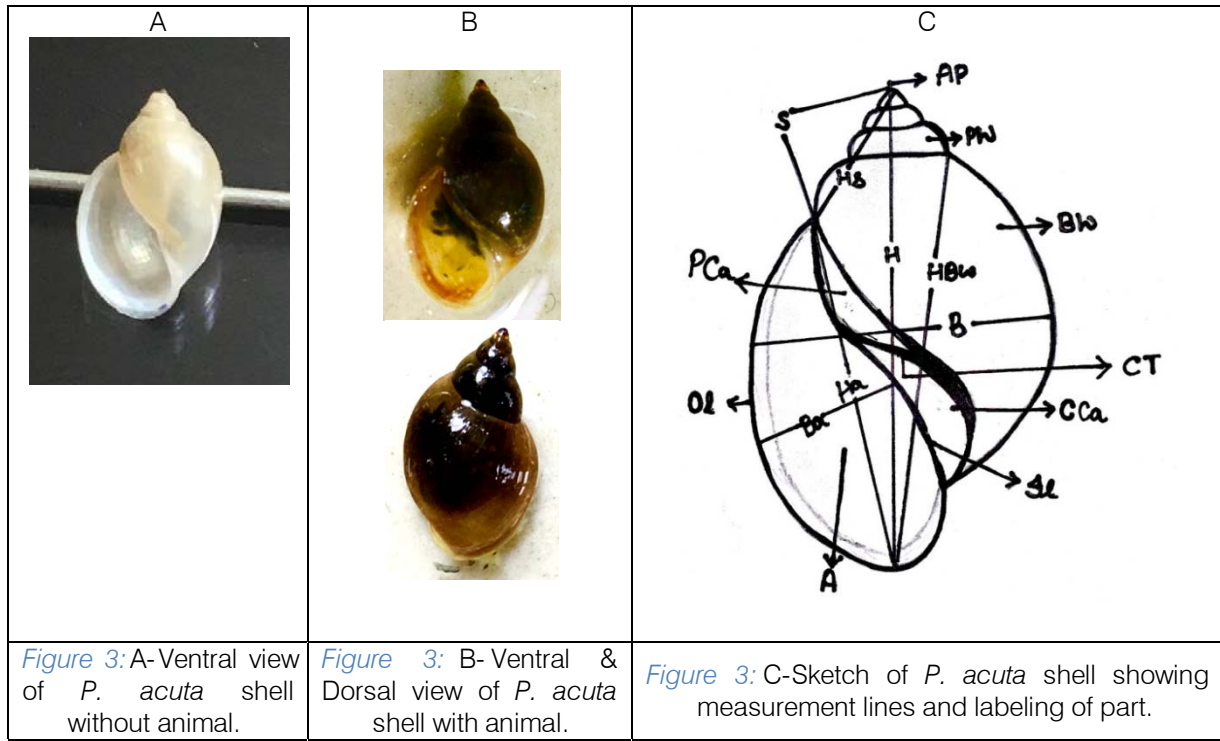


Figure 3: A-Ventral view of *P. acuta* shell without animal.

Figure 3: B-Ventral & Dorsal view of *P. acuta* shell with animal.

Figure 3: C-Sketch of *P. acuta* shell showing measurement lines and labeling of part.

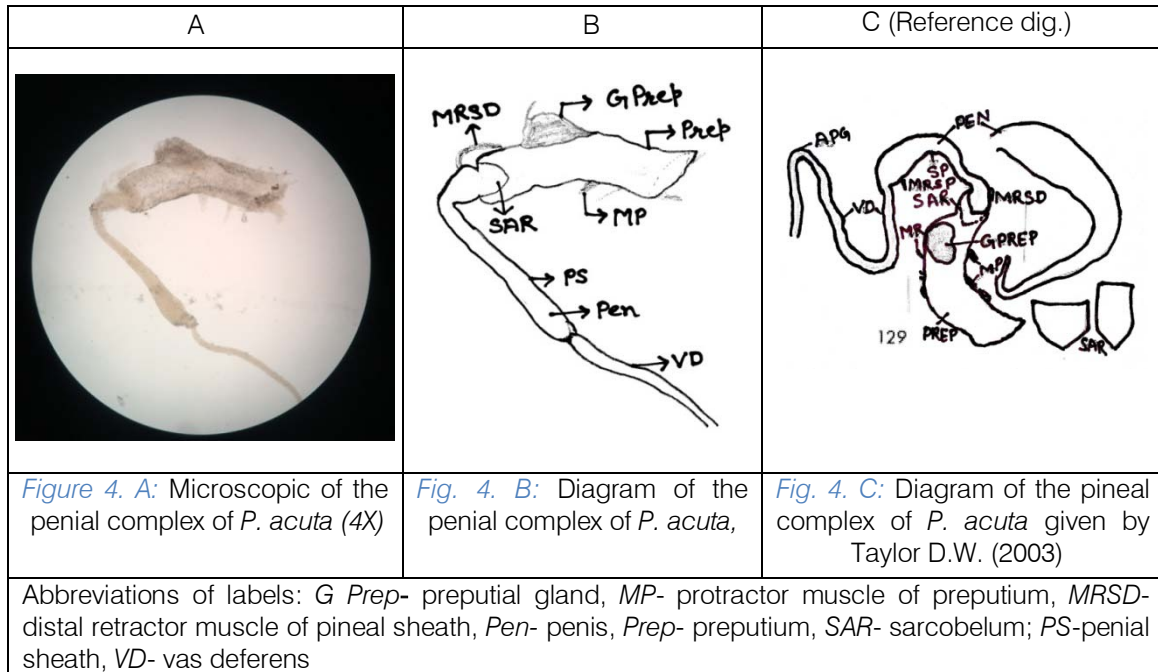
The Number of whorls in different shells of *P. acuta*, collected during the current study ranged from 4 to 5. The height (H) and width (B) of the shells varied from 4mm to 12mm and 2mm to 6 mm respectively. The shells were almost twice the long than broad. The ratio of H & B was 2:1. The height of aperture (Ha) ranged from 2.5mm to 7.5mm while its width (Ba) ranged from

1-3mm and these two parameters were exhibiting the ratio of 2.5:1, means the aperture is two and half times longer than broad. The height of the body whorl (HBw) was found ranging from 3.07-9.2 mm and that of the spire (Hs) from 1.02-3mm. It shows that in *P. acuta* the body whorl is three times longer than its spire. The ratio of length of shells and its aperture was 1.6:1.

Table 1: Measurement of *P. acuta*

S.No.	Height	Value	Width	Measurements	Ratio of parameters	Ratio values
1	Ht. of shell (H)	8 mm	Wd. of shell (B)	4 mm	H:B	2:1
2	Ht. of spire (Hs)	2 mm	-----			
3	Ht. of aperture (Ha)	5 mm	Wd. of aperture (Ba)	2 mm	Ha:Ba	2.5: 1
4	Ht. of body whorl (HBw)	6mm	-----	---	----	----
5	Ht. of penultimate whorl (Hp)	1 mm	-----	---	----	----
6	-----	----	-----	---	HBw: Hs	3:1
7	-----	----	-----	---	H: Ha	1.6:1

c) Anatomical Features



The mantle is colorful with yellow-orange spots that extended out as long pointed extensions over columellar callus, were seen during locomotory movement of *P. acuta*. Tentacles were long, slender, slightly pigmented with eyes situated at their base as clear black spots. Foot, anteriorly bilobed when extended, margin had a few pigmented spots. The Pineal sheath is unipartite and not pigmented. The

preputium is cylindrical, pigmented and having a large, elongate sarcobelum and preputial gland (Fig. 4).

The family has shown its dominance in still waters and slow-moving waters, rich in organic matter while the other sites under study had also shown the presence of *P. acuta* in them, though in very less percentage (Fig. 5).

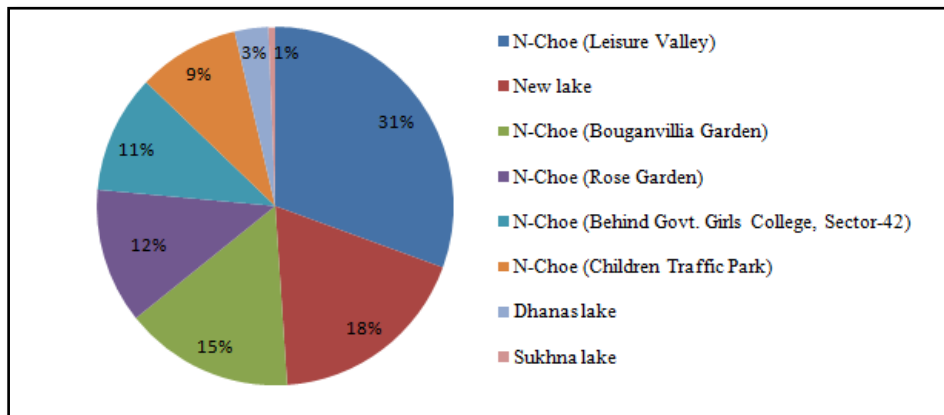


Figure 5: Percentage representation of family Physidae at different sites during the study period (May 2016-2017)

#### IV. DISCUSSION

In the present study, the family Physidae with *P. acuta* had been found for the first time from the freshwater bodies of U.T. Chandigarh. The abundance of snail was found more in slow running freshwater streams with shallow and warm waters (N-Choe) as also reported by Saha *et al.*, (2016). But in the current study the member of the family had also been collected though in less number, from perennial deep-water

bodies too (Sukhna and Dhanas Lake), temporarily fresh deep-water body like New Lake. The shell characteristics were found to be similar to those given by Subbha Rao 1994, Poonam *et al.*, 2013, Rita Bhandari 2016 and many other researchers also. The final confirmation of the species, *P. acuta*, was done by comparing anatomical features especially pineal complex given by Appleton 1989 and Taylor 2003 by comparing the presence and position of the preputial gland.

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## Standardization of Extraction Techniques of Picroside-I and Picroside-II from “Kutki” (*Picrorhiza kurroa* Royle *ex* Benth.)

By Parbat Raj Thani, Yash Pal Sharma, Prashid Kandel & Kriti Nepal

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**Abstract-** Kutki (*Picrorhiza kurroa* Royle *ex* Benth.) is an important medicinal plant used in various herbal drug formulations possessing hepatoprotective activity. The pharmacological importance of this species has been demonstrated due to the presence of iridoid glycosides, such as picroside-I and picroside-II in underground part. In the present study, fresh roots and rhizomes collected from 2600m to 3300m altitude of Himachal Pradesh (Rohtang area) were used and extract yield, phytochemical constituents, namely picroside-I and picroside-II, of water and methanol extracts prepared using four different extraction methods viz. soxhlet, extraction by refluxing, microwave assisted extraction and sonication assisted extraction were compared for the standardization of extraction methods of picroside-I and picroside-II. Sonication assisted extraction for 36 minutes with methanol as solvent yielded 44.269 per cent extract with 6.825 per cent picroside-I and 5.291 per cent picroside-II content, which was better in comparison to other methods regarding time consumption and yield.

**Keywords:** *kutki, microwave assisted extraction, reflux extraction, sonication assisted extraction, soxhlet extraction, standardization.*

**GJSFR-C Classification:** FOR Code: 070599



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# Standardization of Extraction Techniques of Picroside-I and Picroside-II from “Kutki” (*Picrorhiza kurroa* Royle ex Benth.)

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**Abstract-** Kutki (*Picrorhiza kurroa* Royle ex Benth.) is an important medicinal plant used in various herbal drug formulations possessing hepatoprotective activity. The pharmacological importance of this species has been demonstrated due to the presence of iridoid glycosides, such as picroside-I and picroside-II in underground part. In the present study, fresh roots and rhizomes collected from 2600m to 3300m altitude of Himachal Pradesh (Rohtang area) were used and extract yield, phytochemical constituents, namely picroside-I and picroside-II, of water and methanol extracts prepared using four different extraction methods viz. soxhlet, extraction by refluxing, microwave assisted extraction and sonication assisted extraction were compared for the standardization of extraction methods of picroside-I and picroside-II. Sonication assisted extraction for 36 minutes with methanol as solvent yielded 44.269 per cent extract with 6.825 per cent picroside-I and 5.291 per cent picroside-II content, which was better in comparison to other methods regarding time consumption and yield.

**Keywords:** kutki, microwave assisted extraction, reflux extraction, sonication assisted extraction, soxhlet extraction, standardization.

## I. INTRODUCTION

Plants are an important source of bioactive molecules for drug discovery. Isolated bioactive molecules serve as starting materials for laboratory synthesis of drugs as well as a model for the production of biologically active compounds. Phytochemical processing of raw plant materials is essentially required to optimize the concentration of known constituents and also to maintain their activities (Aziz *et al.*, 2003). Extraction is an important step in the itinerary of phytochemical processing for the discovery of bioactive constituents from plant materials.

The general techniques of medicinal plant extraction include maceration, infusion, percolation, digestion, decoction, hot continuous extraction (Soxhlet), aqueous-alcoholic extraction by fermentation, counter-current extraction, microwave assisted solvent extraction, ultrasound assisted solvent extraction (sonication), supercritical fluid extraction and phytonic

extraction (Handa *et al.*, 2008; Co *et al.*, 2012). Selection of a suitable extraction technique is important for the standardization of herbal products as it is utilized in the removal of desirable soluble constituents, leaving out those not required with the aid of the solvents.

Use of green extraction techniques such as Ultrasound Assisted Solvent Extraction (UASE) (Wu *et al.*, 2001; Ahh *et al.*, 2007), Microwave Assisted Solvent Extraction (MASE) (Ganzler *et al.*, 1986a,b) and Supercritical Fluid Extraction (SFE) (Ollanketo *et al.*, 2002) has been rapidly and continuously increasing globally for phytochemical processing of medicinal plants as these techniques are fast as compared to traditional methods. Also, these techniques are environmentally friendly regarding solvent and energy consumption. Yield is also comparable to conventional extraction and in some cases it is even higher. However, extract yield as well as the bioactivities of the extract prepared using different extraction methods have been reported to vary in several studies (Hayouni *et al.*, 2007).

The drug “Kutki” consists of the dried rhizomes and roots of *Picrorhiza kurroa* Royle ex Benth., which is an important alpine herb of Himalayan region growing at an altitudinal range of 3,000 to 5000 m above mean sea level (Kaul and Kaul, 1996; Anonymous, 2001; Vinoth *et al.*, 2010). It is endemic to Western Himalayas extending up to mountains of Yunnan in China (Anonymous, 1969).

It is a well-known drug in the Ayurvedic system of medicine and extensively used in traditional system of medicine in India, China, Tibet, Nepal and Sri Lanka for the treatment of various immune-related diseases (Bhandari *et al.*, 2008). The medicinal importance of *Picrorhiza kurroa* is due to its pharmacological properties like hepatoprotective (Chander *et al.*, 1992), antioxidant (Ansari *et al.*, 1998), antiallergic and antiasthmatic (Dorch *et al.*, 1991), anticancerous activity particularly in liver (Joy *et al.*, 2000), and immunomodulatory, (Gupta *et al.*, 2006).

More than 50 secondary metabolites have been reported from the plant *Picrorhiza kurroa* which includes iridoid glycosides, cucurbitacins and phenolic compounds. The pharmacological importance of *Picrorhiza kurroa* has been demonstrated due to the presence of iridoid glycosides, such as picroside-I and picroside-II (Rastogi *et al.*, 1996).

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With increasing demand for herbal medicinal plants, and natural products for health care all over the world, herbal manufacturers aim at using the most appropriate extraction technologies to produce extracts of defined quality and quantity with least batch to batch variation. Standardization of extraction procedures contributes significantly to the final quality and quantity of the product. The selection of method to isolate active components with the best yield and high purity with minimum inputs is mainly dependent on the nature of compounds and raw material which to be processed (Kothari *et al.*, 2009).

The basic parameters influencing the quality of an extract are plant parts used as starting material, the solvent used for extraction and extraction procedure (Ncube *et al.*, 2008).

Keeping in view to find the correlation between different extraction methods, extraction yield, total phyto constituents picroside-I and picroside-II and to standardize the extraction techniques, the present investigation was carried out.

## II. MATERIALS AND METHODS

### a) Plant material and extract preparation

Fresh roots and rhizomes of *Picrorhiza kurroa* were collected from 2600m to 3300m altitude of Himachal Pradesh (Rohtang area) in 2012 and harvested material was washed with water to remove the soil and other adhering material and then cut into small pieces and dried under shade. The air-dried material was then ground to form uniform powdered material. Accurately weighed air-dried and powdered plant material (2g) each was subjected to extract using five extraction techniques, namely soxhlet, reflux, sonication, microwave with methanol and methanol: water (90:10).

For soxhlet extraction, thimble having 2g powdered plant material prepared and extracted with methanol for 4 hours, 8 hours, 12 hours, 16 hours, 20 hours and 24 hours separately at 100° C on water bath. After extraction, the solvent was distilled off and the residue was dried to a constant weight and yield of extract was recorded.

For refluxing, 2g of powdered plant material was mixed with methanol in a round bottom flask and refluxed for 2 hours, 4 hours, 6 hours, 8 hours 10 hours and 12 hours separately at 100° C on water bath. Liquid extracts obtained were separated from the solid residue by vacuum filtration and the solvent was distilled off and dried to a constant weight and yield of extract was recorded.

For ultrasound assisted sonication extraction, air dried powdered plant material (2g) was mixed with

methanol in a flask and extracted for 20 minutes, 24 minutes, 28 minutes, 32 minutes, 36 minutes and 40 minutes separately. Temperature was maintained at 35°C ± 1°C and sonication power was 120 MHz. After extraction, the material was filtered and then solvent from the filtrate was distilled off and the residue was dried to a constant weight and yield of extract was recorded.

Similarly, for microwave assisted solvent extraction, powdered plant material (2g) was mixed with methanol and methanol: water (90:10) separately in the flask and extracted for 20 minutes, 24 minutes, 28 minutes, 32 minutes, 36 minutes and 40 minutes separately. The domestic microwave oven of IFB brand model 30SC3 was used for microwave assisted extraction. Microwave output power was maintained 180 watts (20 % out of 900 watts). The supernatant was similarly preceded as described in UASE to get dried MASE extract of *Picrorhiza kurroa*.

The yield of the extract obtained from the different extraction methods was calculated by using the following formula.

$$\text{Yield (\%)} = \frac{\text{The weight of the residue obtained}}{\text{The weight of the plant material taken}} \times 100$$

### b) Quantitative estimation of picroside-I and picroside-II in extracts using high-performance liquid chromatography (HPLC)

Picroside-I and picroside-II were quantified in the extracts using HPLC (Water's binary HPLC unit with Waters HPLC pump 515 and dual λ absorbance detector 2487) on column Sunfire C-18 (4.6 × 250 mm, 5μm). The mobile phase consisted of methanol: water (40: 60) with the flow rate of 0.9 ml/min. The temperature was maintained 24° ± 1° C.

Each dried extract was 1000 times diluted with mobile phase, filtered through the membrane filter and 20μl of each sample was injected in HPLC, and Area Under Curve for peaks of picroside-I and picroside-II was recorded at 270 nm wavelength on the basis of retention time with the comparison of the retention time of standard picroside-I and picroside-II, their contents in the extracts were quantified on the basis of area of peak.

Percentage of Picroside-I and picroside-II in each sample was calculated by using the following formula where percent purity of the standard compound was taken as 95%:

$$\text{Picrosides content(\%)} = \frac{\text{Test Area}}{\text{Standard Area}} \times \frac{\text{Wt. of Standard}}{\text{Standard Dilution}} \times \frac{\text{Test Dilution}}{\text{Test Weight}} \times 100 \times \text{Percent Purity}$$

### III. RESULTS AND DISCUSSION

Biologically active compounds usually occur in low concentration in plants. An extraction technique is that which can obtain extracts with high yield and with minimal changes to the functional properties of the extract required (Quispe Candori *et al.*, 2008). Several studies have reported variations in the biological activities of extracts prepared using different extraction techniques. Therefore, it is necessary to select the suitable extraction method as well as solvent based on sample matrix properties, chemical properties of the analytes, matrix-analyte interaction, efficiency and desired properties (Hayouni *et al.*, 2007; Ishida *et al.*, 2001).

In conventional extraction, heat is transferred through convection and conduction from the surface, here, the extractability of solvents depends mainly on the solubility of the compound in the solvent, the mass transfer kinetics of the product and the strength of solute/matrix interaction with corresponding limitations on heat and mass diffusion rate. Ultrasound assisted solvent extraction is a process that uses high intensity, high frequency sound wave and solvents to extract targeted compounds from various matrices. Physical and chemical properties of the materials subjected to ultrasound are altered due to the propagation and interaction of sound waves as they disrupt the plant cell walls, thereby, facilitating the release of extractable compounds and enhancing mass transport of solvent from the continuous phase into plant cells. Microwave energy and solvents are used for the extraction of targeted compounds from plant matrices in the case of microwave assisted solvent extraction. Highly localized temperature and pressure can cause selective migration of targeted compounds from the matrices to the surroundings at a more rapid rate. Recoveries are similar or better in both UASE and MASE as compared to conventional extraction. However, reduced extraction time and solvent consumption are the main advantages of UASE and MASE. Although, extraction of bioactive compounds from the plants has been extensively investigated using conventional solvent extraction, the present investigation was undertaken to study the effect of extraction duration on phytochemical extraction through different methods on yield and phytochemical qualities of *P. kurroa* and after standardization of individual method, all the methods were compared so as to find out the best extraction method with maximum picroside-I and picroside-II content.

Extract yield from roots and rhizomes of *P. kurroa* prepared by soxhlet, refluxing, UASE and MASE methods under different duration using methanol and methanol-water, and the present quantity of phytochemical constituents (picroside-I and picroside-II) is summarized in Table 1 to 5 and the comparison

among different methods of extraction is presented in Table 6.

In soxhlet extraction, 4 hours, 8 hours, 12 hours, 16 hours, 20 hours and 24 hours were used for the extraction of raw material, where extraction up to 12 hours was found the best time of extraction with maximum extraction yield (28.931%), picroside-I (5.937%) and picroside-II (5.212%).

In reflux extraction method, 2 hours, 4 hours, 6 hours, 8 hours, 10 hours and 12 hours were used for the extraction of raw materials, where 6 hours was found the best time of extraction with maximum extraction yield (22.713%), picroside-I (5.991%) and picroside-II (5.120%).

In sonication method, 20 minutes, 24 minutes, 28 minutes, 32 minutes, 36 minutes and 40 minutes were used for the extraction of raw materials, where 36 minutes was the best time of extraction with maximum extraction yield (44.269%), picroside-I (6.825%) and picroside-II (5.291%).

Similarly, in microwave, 100% methanol and methanol: water (90:10) method, similar time periods (20 minutes, 24 minutes, 28 minutes, 32 minutes, 36 minutes and 40 minutes) were used to extract raw materials, where 28 minutes was found the best time of extraction with maximum extract yield and phytochemical content for both methods, however the quantity of extract yield and phytoconstituents varied. In former, extract yield recorded (23.488%), picroside-I (2.642%) and picroside-II (2.192%) and in later, extract yield (15.346%), picroside-I (1.878%) and picroside-II (1.489%) was recorded. It showed improvement of yield and bioactive content in case of the method where solvent was only methanol instead of the mixture of methanol and water.

The comparison carried out among different extraction methods after standardization of extraction duration under different extraction methods showed sonication assisted extraction method to be the best method for 36 minutes extraction with maximum total extract (44.269%), maximum picroside-I content (6.825%) and maximum picroside-II content (5.291%).

The similar results have been reported by Sun *et al.*, (2011), Jadhav *et al.*, (2009), Xia *et al.*, (2006), Smelcerovic *et al.*, (2006), and Bimkr *et al.*, (2013) while comparison was carried out among various extraction methods for the various phytoconstituents from various plant materials.

**Table 1:** Effect of Extraction Duration on Phytochemical Extraction through Soxhlet Extraction Method

Extraction duration	Total extract%	Picroside-I (%)	Picroside-II (%)
4 hours	24.225	5.515 (2.552)	4.774 (2.403)
8 hours	25.486	5.833 (2.614)	4.850 (2.418)
12 hours	28.913	5.975 (2.641)	5.212 (2.492)
16 hours	29.425	6.052 (2.655)	5.227 (2.495)
20 hours	29.988	6.062 (2.657)	5.228 (2.496)
24 hours	30.000	6.062 (2.657)	5.223 (2.494)

(Values in parentheses are transformed values using  $\sqrt{x + 1}$  transformed values)

**Table 2:** Effect of Extraction Duration on Phytochemical Extraction through Reflux Extraction Method

Extraction duration	Total extract (%)	Picroside-I (%)	Picroside-II (%)
2 hours	6.200	5.663 (2.581)	4.481 (2.314)
4 hours	8.175	5.805 (2.609)	4.538 (2.353)
6 hours	22.713	5.991 (2.644)	5.120 (2.474)
8 hours	32.688	6.011 (2.648)	5.125 (2.475)
10 hours	34.050	5.945 (2.635)	5.124 (2.474)
12 hours	34.988	5.923 (2.631)	5.116 (2.473)

(Values in parentheses are transformed values using  $\sqrt{x + 1}$  transformed values)

**Table 3:** Effect of Extraction Duration on Phytochemical Extraction through Sonication Extraction Method

Extraction duration	Total extract (%)	Picroside-I (%)	Picroside-II (%)
20 minutes	33.013	5.826 (2.610)	4.702 (2.387)
24 minutes	38.018	6.459 (2.732)	5.011 (2.452)
28 minutes	40.994	6.621 (2.760)	5.235 (2.497)
32 minutes	42.533	6.799 (2.792)	5.286 (2.507)
36 minutes	44.269	6.825 (2.797)	5.291 (2.508)
40 minutes	45.135	6.825 (2.797)	5.293 (2.508)

(Values in parentheses are transformed values using  $\sqrt{x + 1}$  transformed values)

**Table 4:** Effect of Extraction Duration on Phytochemical Extraction through microwave, Methanol: Water (90:10) Extraction Method

Extraction duration	Total extract(%)	Picroside-I (%)	Picroside-I (%)
20 min	4.668	1.509(1.584)	1.285(1.512)
24 min	5.584	1.685(1.639)	1.398(1.548)
28 min	15.346	1.878(1.696)	1.489(1.578)
32 min	17.495	1.853(1.689)	1.433(1.560)
36 min	21.553	1.802(1.674)	1.398(1.548)
40 min	25.54	1.776(1.666)	1.361(1.537)

(Values in parentheses are transformed values using  $\sqrt{x + 1}$  transformed values)

**Table 5:** Effect of Extraction Duration on Phytochemical Extraction through Microwave, Methanol 100% Extraction Method

Extraction duration	Total extract (%)	Picroside-I (%)	Picroside-II (%)
20 minutes	5.916	1.735 (1.654)	1.435 (1.560)
24 minutes	9.306	2.613 (1.901)	1.993 (1.730)
28 minutes	23.488	2.642 (1.908)	2.192 (1.786)
32 minutes	24.742	2.554 (1.885)	2.045 (1.745)
36 minutes	30.316	2.478 (1.865)	1.977 (1.725)
40 minutes	34.425	2.415 (1.848)	1.798 (1.672)

(Values in parentheses are transformed values using  $\sqrt{x + 1}$  transformed values)

**Table 6:** Effect of Different Extraction Methods on Total Extract, Picroside-I and Picroside- II Content (%)

Extraction method	Extraction time	Total extract %	Picroside-I %	Picroside-II%
Soxhlet extraction	12 hours	28.913	5.975 (2.641)	5.212 (2.492)
Reflux extraction	6 hours	22.713	5.991 (2.644)	5.120 (2.474)
Sonication	36 minutes	44.269	6.825 (2.797)	5.291 (2.508)
Microwave, methanol: water (90:10)	28 minutes	15.346	1.878 (1.696)	1.489 (1.578)
Microwave, 100% methanol	28 minutes	23.488	2.642 (1.908)	2.192 (1.786)

(Values in parentheses are transformed values using  $\sqrt{x+1}$  transformed values)

#### IV. CONCLUSION

The standardization of the extraction process is very important for the isolation of bioactive compound. In the present work, the results indicate that ultrasound assisted sonication extraction method can be viable alternative method to extract higher yield along with phytoconstituents (picroside-I and picroside-II). The result also reveals that the methanol can be used as a suitable solvent rather than the mixture of methanol-water for extraction of polar compounds.

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## Isolation and Identification of Pathogenic Microorganisms from Houseflies

By Ibrahim, A. W., Ajiboye. T. O., Akande T. A. & Anibaba, O. O.

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**Abstract-** The housefly, *Musca domestica*, is a fly of the suborder *Cyclorrhapha*. It is an important vector for transmission of pathogenic microorganisms including bacteria, fungi and parasites. A total of 27 houseflies were collected, 9 each from dumpsite, canteen and indoor premise with the use of insect traps. The bacteria isolates obtained are: *Escherichia coli*, *Salmonella* species, *Pseudomonas* species, *Shigella* species, *Klebsiella* species, *Staphylococcus* species, *Streptococcus* species, *Bacillus* species and *Proteus* species. The fungi isolated are *Aspergillus* species, *Penicillium* species, *Alternaria* species and *Fusarium* species. The parasites obtained include *Gardia lamblia*, *Entamoeba histolytica*, *Enterobius vermicularis* and *Strongyloides* species. Dumpsite had the highest percentage occurrence of microorganisms, followed by indoor and food canteen which had the lowest percentage occurrence. The presence of these pathogenic microorganisms in food canteens, indoor toilets and dumpsite implied a possible risk of transmission of the pathogens from the houseflies to humans thereby causing diseases; hence, the need to step up control measures against the insects.

**Keywords:** housefly; *musca domestica*, bacteria, fungi, parasites, food canteens, toilets and dumpsites.

**GJSFR-C Classification:** FOR Code: 069999



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# Isolation and Identification of Pathogenic Microorganisms from Houseflies

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**Abstract-** The housefly, *Musca domestica*, is a fly of the suborder *Cyclorrhapha*. It is an important vector for transmission of pathogenic microorganisms including bacteria, fungi and parasites. A total of 27 houseflies were collected, 9 each from dumpsite, canteen and indoor premise with the use of insect traps. The bacteria isolates obtained are: *Escherichia coli*, *Salmonella* species, *Pseudomonas* species, *Shigella* species, *Klebsiella* species, *Staphylococcus* species, *Streptococcus* species, *Bacillus* species and *Proteus* species. The fungi isolated are *Aspergillus* species, *Penicillium* species, *Alternaria* species and *Fusarium* species. The parasites obtained include *Giardia lamblia*, *Entamoeba histolytica*, *Enterobius vermicularis* and *Strongyloides* species. Dumpsite had the highest percentage occurrence of microorganisms, followed by indoor and food canteen which had the lowest percentage occurrence. The presence of these pathogenic microorganisms in food canteens, indoor toilets and dumpsite implied a possible risk of transmission of the pathogens from the houseflies to humans thereby causing diseases; hence, the need to step up control measures against the insects.

**Keywords:** housefly; *musca domestica*, bacteria, fungi, parasites, food canteens, toilets and dumpsites.

## I. INTRODUCTION

The housefly, *Musca domestica* is the most common fly species found in habitations such as refuse dumps, toilets, domestic waste bins and other areas of poor sanitary conditions. Houseflies enter several places, including contaminated premises because of their own biologic habits for feeding (Service, 2000). It is not only a nuisance pest but also acts as an important vector for lots of pathogenic microorganism including bacteria, protozoa, fungi and viruses among humans and animals (Hussein and John, 2017). Houseflies transmit these disease agents by means of different parts of their bodies (hairs body, appendages and mouth parts) and secretions (regurgitates and faeces) (Babak *et al.*, 2008).

Furthermore, they enhance the spread of diseases such as cholera caused by *Vibrio cholerae*, typhoid and paratyphoid fever by *Salmonella typhi* and *Salmonella paratyphi*, bacillary dysentary caused by *Shigella* species, tuberculosis caused by *Mycobacterium tuberculosis*, anthrax caused by *Bacillus anthracis* and many others amongst human's population as well as their livestock (Isabel, 2015).

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Some of the fungal diseases transmitted by houseflies include: Aspergillosis caused by *Aspergillus* species, Penicilliosis caused by *Penicillium* species, Onychomycosis caused by *Fusarium* species, Alternariosis caused by *Alternaria* species, etc (Davari *et al.*, 2012). Houseflies have been identified as vectors of protozoan parasites that causes diseases such as Sarcocystis caused by *Sarcocystis* species, Toxoplasmosis caused by *Toxoplasma gondii*, Isosporiasis caused by *Isospora* species, Giardiasis caused by *Giardia* species, Amoebiasis caused by *Entamoeba histolytica*, etc (Thaddeus *et al.*, 2005). Pathogenic organisms are picked up by flies from garbage, sewage and other sources of filth, and then transferred on their mouth parts and other body parts through their vomits, faeces and contaminated external body parts to human and animal food (Babak *et al.*, 2008). Macovei and Zurek (2006) have reported which houseflies in food-handling and serving facilities harbour and may have the capacity to transfer antibiotic-resistant and potentially virulent strains of pathogenic microorganisms.

*Musca domestica* is capable of carrying a variety of bacteria, viruses, fungi and parasitic diseases over its body appendages and can therefore pose a threat to the societal health, but despite the awareness of the dangers posed by houseflies, the inability to maintain a good sanitation leads, improper handling of food, indiscriminate refuse dumping and little or no care of toilet facilities have led to an increase in the population of houseflies. Therefore, the aim of this study was to isolate and identify bacteria, fungi and parasites picked up by houseflies in the food handling setting, dumpsite and indoor premises.

## II. MATERIALS AND METHODS

### a) Sample collection

A total of 27 houseflies were collected, 9 samples each, from dumpsite, canteens and indoor premises in Ilorin, Kwara state. The method of collection was by the use of an insect trap to capture the houseflies, and then the houseflies were killed by exposure to chloroform for few minutes in the traps and then placed in sterile universal bottles individually, properly labeled and transported immediately to the laboratory.

#### b) Preparation of media

All media were prepared in the Erlenmeyer flask according to the manufacturer's instructions. The media used were MacConkey agar, Nutrient agar, Potato dextrose agar, Citrate agar, Sulphide Indole Motility (SIM) agar and Triple Sugar Iron (TSI) agar. The media used were sterilized in the autoclave at 121°C for 15 minutes.

#### c) Isolation and maintenance of the isolates

The houseflies were each placed in a test tube containing 1.0ml sterile normal saline by using a pair of forceps, the flies were gently rinsed by stirring with a glass rod in order to wash the microbial flora on the external parts of the houseflies into the normal saline then a drop of the normal saline from each tube was inoculated on Nutrient, Potato dextrose and MacConkey agar plates by streaking with the use of a flame sterilized inoculating loop, this was done in duplicates and around the flame to maintain aseptic condition.

The remnant was centrifuged and decanted to obtain the concentrate which was later used to make a wet mount and was examined using the 10X and 40X objective of the microscope for the presence of parasites.

The houseflies were then collected from the test tubes and washed in ethyl alcohol to decontaminate their surfaces. They were then washed in normal saline to wash off excess alcohol that may affect the internal microbial flora during dissection. The flies were then each placed on a sterile slide where they were dissected under a dissecting microscope. The guts were obtained and placed in test tubes containing 1.0ml of normal saline and homogenized. The resulting mixture was cultured and incubated in the same way as the external body surface.

Inoculated plates were then incubated in an inverted position aerobically at 37°C for 24 hours. After 24 hours of incubation, distinct colonies were selected randomly and subcultured on nutrient agar and potato dextrose agar plates to obtain pure cultures. This is then incubated aerobically in an inverted position at 37°C for 24 hours. For maintenance of the isolates, 24 hours pure culture of the isolates were transferred to nutrient agar and potato dextrose agar slant and stored at 0-4°C.

#### d) Identification of the bacterial isolates

The cultured bacterial isolates were identified using morphological characteristics namely: Colonial morphology, Gram staining and Endospore staining and biochemical characteristics namely: Catalase test, Oxidase test, Coagulase test, Citrate utilization test, sugar fermentation test (lactose, glucose and sucrose), hydrogen sulphide production, indole production and motility test as described by Cheesebrough (2006) and Fawole and Osho (2007).. Triple Sugar Iron (TSI) agar and Hydrogen Sulphide, Indole and Motility (SIM) agar were used for sugar fermentation, H<sub>2</sub>S production,

Indole production and Motility tests respectively. The bacterial isolates were identified based on their biochemical characteristics using Bergey's Manual of Determinative Bacteriology (Bergey and John, 1994).

#### e) Identification of the fungal isolates

The cultured fungal isolates were identified using colonial morphology and microscopically using Lactophenol blue staining.

#### f) Identification of the parasites

The parasites were identified microscopically by making wet mount of the parasites and examining them using the 10X and 40X objective of the microscope for the presence of parasites.

### III. RESULTS

#### a) Bacteria identification

Out of the 30 bacteria isolates obtained in this study, twenty three (23) of the bacteria were Gram negative (nineteen (19) from the external surface of the houseflies and four (4) from the intestinal guts of the houseflies) while seven (7) of the bacteria isolates were Gram positive (external surface of the houseflies). The bacterial isolates were further characterized based on the morphological and biochemical identifications as shown in table 1, 2 and 3. In all, nine (9) bacterial genera were identified.

The Gram negative bacteria isolates from the external surface of the houseflies were identified as *Escherichia coli* (36.8%), *Salmonella* species (26.3%), *Pseudomonas* species (5.3%), *Shigella* species (26.3%) and *Klebsiella* species (5.3%) as shown in figure 1.

The Gram positive bacteria isolates from the external surface of the houseflies were identified as *Staphylococcus* species (42.9%), *Streptococcus* species (28.6%) and *Bacillus* species (28.6%) as shown in figure 2.

The Gram negative bacteria isolates from the internal surface of the houseflies were identified as *Escherichia coli* (50%), *Klebsiella* species (25%) and *Proteus* species (25%) as shown in figure 3.

The microorganisms isolated from the different study sites were compared to each other, Canteen (26.7%), dumpsite (43.3%) and indoor (30%) as shown in figure 4.



**Table 1:** Morphological and Biochemical Identification for the Gram Negative Bacteria Isolated from the External Surface of the Houseflies

Isolates	Gram reaction	Morphology	Catalase	Oxidase	H <sub>2</sub> S	Indole	Motility	Citrate	Glucose	Sucrose	Lactose	Gas	Probable organisms
CA1	-	Rods	+	-	-	+	+	-	A	A	A	+	<i>Escherichia coli</i>
CA2	-	Rods	+	-	+	-	+	-	A	-	-	-	<i>Salmonella</i> sp.
CA4	-	Rods	+	-	+	-	+	-	A	-	-	-	<i>Salmonella</i> sp.
CA5	-	Rods	+	+	-	-	+	+	-	-	-	-	<i>Pseudomonas</i> sp.
CB1	-	Rods	+	-	-	+	-	-	-	-	-	-	<i>Shigella</i> sp.
CB2	-	Rods	+	-	+	-	+	-	A	-	-	-	<i>Salmonella</i> sp.
DA2	-	Rods	+	-	-	+	-	-	-	-	-	-	<i>Shigella</i> sp.
DB1	-	Rods	+	-	-	-	-	+	A	A	A	+	<i>Klebsiella</i> sp.
DB2	-	Rods	+	-	-	+	+	-	A	A	A	+	<i>Escherichia coli</i>
DB3	-	Rods	+	-	-	+	-	-	-	-	-	-	<i>Shigella</i> sp.
DB5	-	Rods	+	-	-	+	+	-	A	A	A	+	<i>Escherichia coli</i>
DC1	-	Rods	+	-	+	-	+	-	A	-	-	-	<i>Salmonella</i> sp.
DC4	-	Rods	+	-	-	+	+	-	A	A	A	+	<i>Escherichia coli</i>
DC5	-	Rods	+	-	-	+	-	-	-	-	-	-	<i>Shigella</i> sp.
IA1	-	Rods	+	-	-	+	+	-	A	A	A	+	<i>Escherichia coli</i>
IA2	-	Rods	+	-	+	-	+	-	A	-	-	-	<i>Salmonella</i> sp.
IB1	-	Rods	+	-	-	+	+	-	A	A	A	+	<i>Escherichia coli</i>
IC2	-	Rods	+	-	-	+	-	-	-	-	-	-	<i>Shigella</i> sp.
IC3	-	Rods	+	-	-	+	+	-	A	A	A	+	<i>Escherichia coli</i>

**Table 2:** Morphological and Biochemical Identification for the Gram Positive Bacterial Isolated from the External Surface of the Houseflies

Isolates	Gram reaction	Morphology	Spore	Catalase	Coagulase	H <sub>2</sub> S	Indole	Motility	Citrate	Glucose	Lactose	Sucrose	Gas	Probable organisms
CA3	+	Cocci	-	+	+	-	-	-	+	A	A	A	-	<i>Staphylococcus</i> sp.
DA1	+	Cocci	-	-				-	-	A	A	A	-	<i>Streptococcus</i> sp.
DB4	+	Rods	+	+			-	+	+	A	-	A	-	<i>Bacillus</i> sp.
DC2	+	Cocci	-	+	+	-	-	-	+	A	A	A	-	<i>Staphylococcus</i> sp.
DC3	+	Cocci	-	+	+	-	-	-	+	A	A	A	-	<i>Staphylococcus</i> sp.
IB2	+	Rods	+	+			-	-	+	A	-	A	-	<i>Bacillus</i> sp.
IC1	+	Cocci	-	-		-	-	-	-	A	A	A		<i>Streptococcus</i> sp.

**Table 3:** Morphological and Biochemical Identification for the Gram Negative Bacteria Isolated from the Intestinal guts of the Houseflies

Isolates	Gram reaction	Morphology	Catalase	Oxidase	H <sub>2</sub> S	Indole	Motility	Citrate	Glucose	Sucrose	Lactose	Gas	Probable organisms
C1	-	Rods	+	-	-	+	+	-	A	A	A	+	<i>Escherichia coli</i>
D1	-	Rods	+	-	-	-	-	+	A	A	A	+	<i>Klebsiella sp.</i>
I1	-	Rods	+	-	+	-	+	+	A	-	-	+	<i>Proteus sp.</i>
I2	-	Rods	+	-	-	+	+	-	A	A	A	+	<i>Escherichia coli</i>

**KEY**

C= Canteen

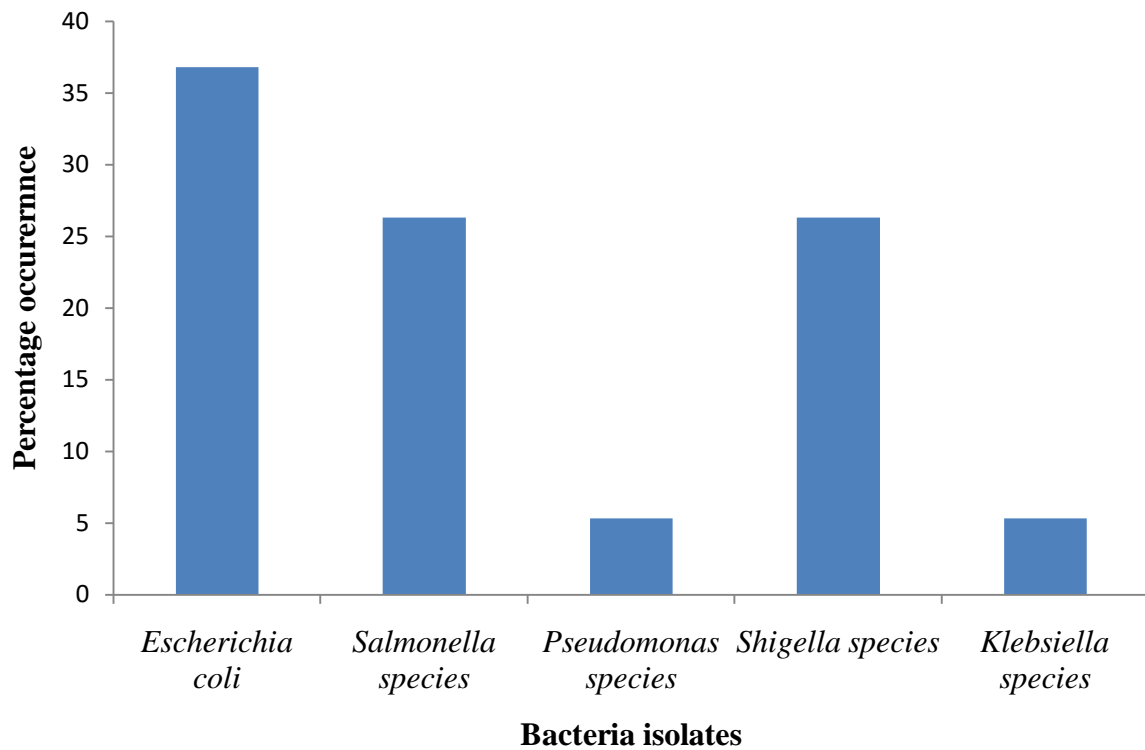
D= Dumpsite

I= Indoor

+ = Positive

- = Negative

A = Acid production

**Figure 1:** Percentage occurrence of Gram negative organisms from the external surface of the houseflies

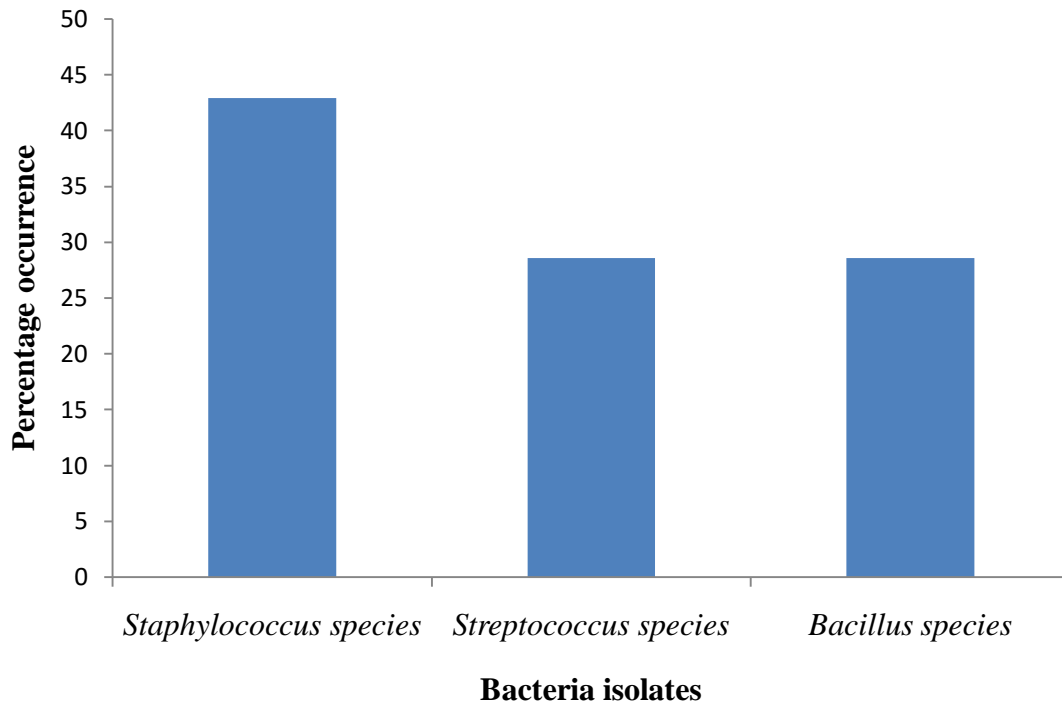


Figure 2: Percentage occurrence of Gram positive bacteria from the external surface of the houseflies

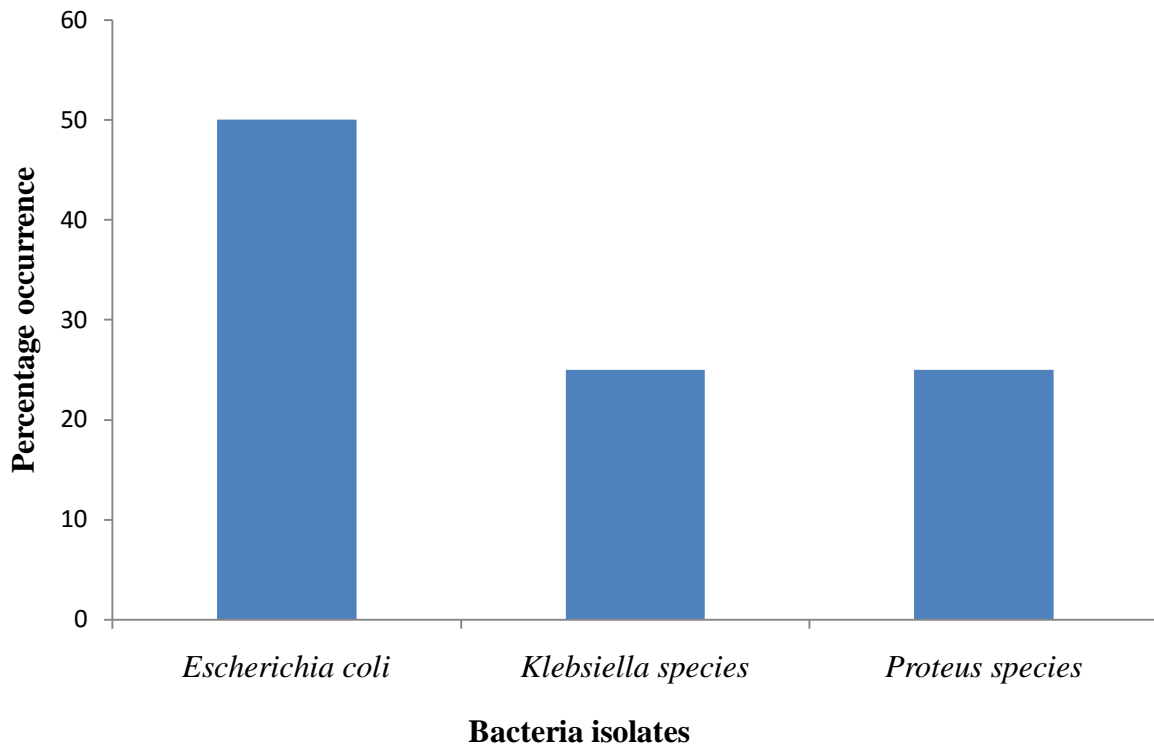


Figure 3: Percentage occurrence of bacteria isolates from the intestinal guts of the houseflies

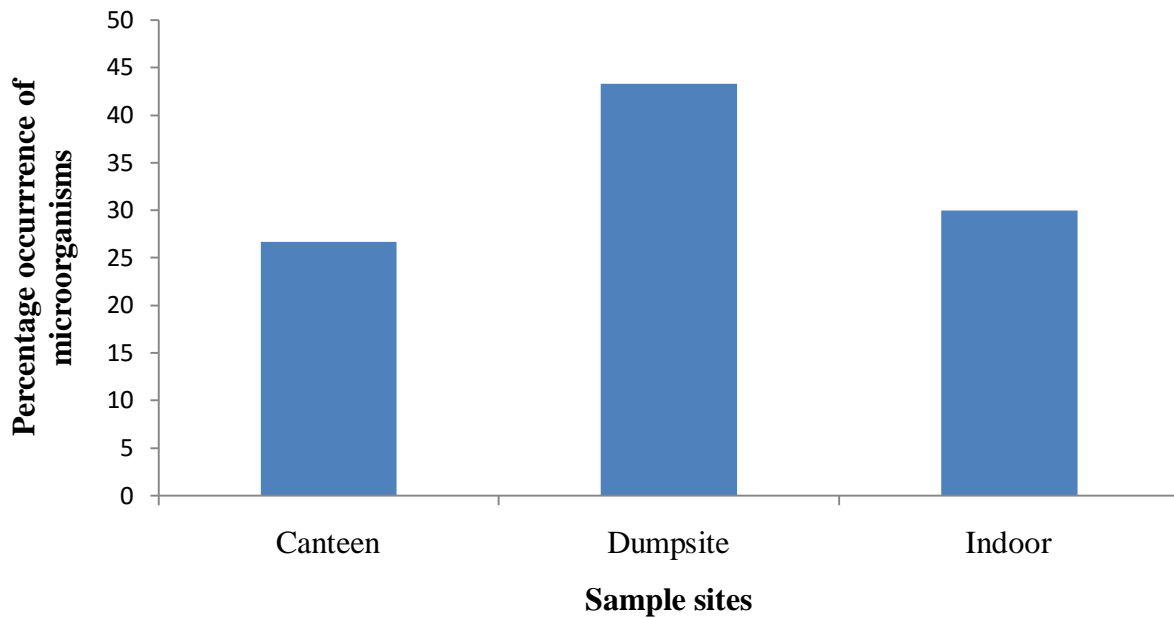


Figure 4: Percentage occurrence of microorganisms in the different sample sites

#### IV. FUNGI IDENTIFICATION

Out of 10 fungi isolates, three (3) were *Aspergillus* species, four (4) were *Penicillium* species, one (1) was *Alternaria* species and two (2) were

*Fusarium* species as shown in figure 5. In all, four (4) fungal genera were isolated. They were identified based on microscopic examination.

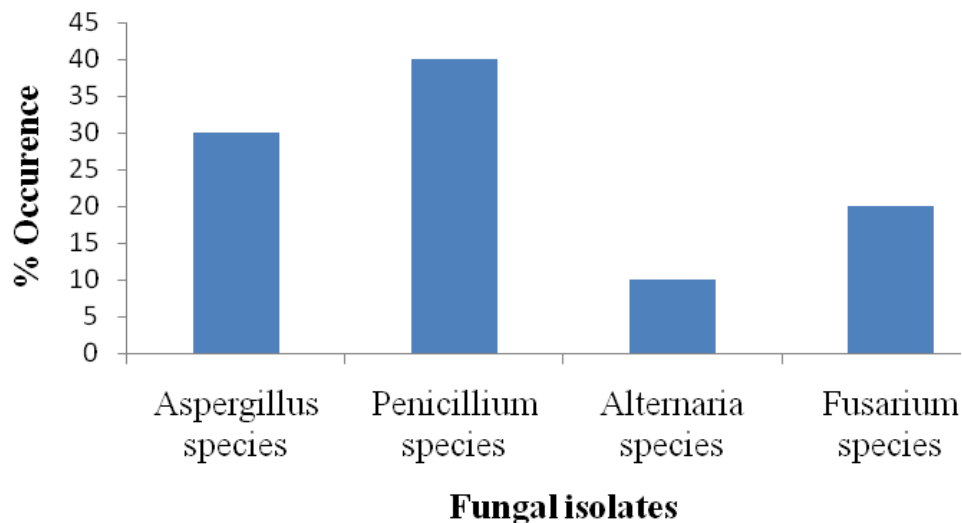


Figure 5: Percentage occurrence of fungi isolates from the external surface of the houseflies

#### V. PARASITES IDENTIFICATION

A total of twenty two (22) parasites were identified based on microscopic examination, eight (8) were *Entamoeba histolytica* (36.36%), eight (8) were *Giardia lamblia* (36.36%), two (2) were *Enterobius vermicularis* (9.09%) and four (4) were *Strongyloides* spp (18.18%) as shown in table 4 and figure 6. In all, four (4) parasites were identified.

Table 4: Parasites identified and their percentage occurrence

Species of parasites	Class	No. Isolated	Percentage
<i>Entamoeba histolytica</i>	Protozoa	8	36.36
<i>Giardia lamblia</i> cyst	Protozoa	8	36.36
<i>Enterobius vermicularis</i> egg	Nematode	2	9.09
<i>Strongyloides</i> spp. egg	Nematode	4	18.18

## VI. DISCUSSION

The result of this study confirmed the mechanical transmission of pathogenic microorganisms by housefly, *Musca domestica*. Some of the bacteria genera isolated in this study such as *Klebsiella* species, *Bacillus* species, *Staphylococcus* species and *Pseudomonas* species correlates with the findings of Hamid *et al.*, (2012). The bacteria species isolated from the outer parts of the houseflies include *Escherichia coli* (which was the most frequently occurring), *Staphylococcus* species, *Shigella* species, *Streptococcus* species, *Salmonella* species, *Klebsiella* species and *Pseudomonas* species is similar to the findings of Mawak and Olukose (2006) and Babak *et al.*, (2008). The isolation of *Salmonella* is quite notable because salmonellosis is currently regarded as one of the most common food – borne zoonotic infections in the world causing diarrhea (Songe *et al.*, 2017). From the intestinal parts of the houseflies, *Escherichia coli*, was the most frequently occurring and *Klebsiella* species and *Proteus* species being the least frequently occurring is in agreement with the findings of Mawak and Olukose (2006). The isolation of some bacteria from houseflies in this study not only corroborate the findings of some earlier studies, but also raises the possibilities of spread of antibiotic resistant pathogens as some of the similar pathogens isolated in other studies have been shown to have antibiotic resistance. Hemmatinezhad *et al.*, (2015) reported the isolation of antimicrobial resistant strain of *Pseudomonas aeruginosa* in Iran.  $\beta$ -lactamase-producing *Escherichia coli* was isolated from houseflies in Spanish broiler farms (Solar-Gines *et al.*, 2015) highlighting the potential contribution of houseflies to the rise and spread of virulence and resistance genes into different ecological niches. *Aspergillus* species, *Penicillium* species, *Fusarium* species and *Alternaria* species were isolated in this study and this is similar with the findings of Davari *et al.*, (2012). The detection of *Giardia lamblia*, *Entamoeba histolytica*, *Enterobius vermicularis* and *Strongyloides stercoralis* correlates with Mawak and Olukose (2006).

## VII. CONCLUSION

The presence of these pathogens which include *Escherichia coli*, *Staphylococcus* species, *Klebsiella* species, *Entamoeba histolytica*, *Giardia lamblia*, *Aspergillus* species, etc. in houseflies found in food canteens, dumpsite and indoor coupled with their

intimacy with man and their highly motile nature implies a possible risk of transmission of the pathogens from the houseflies to humans thereby causing diseases. To prevent this, control measures against the houseflies must be employed such as enforcing strict legislative standards to ensure hygienic condition of places like food canteens, public toilets and dumpsite, proper hygiene and environment sanitation should be practised and the public should be enlightened on the dangers of poor sanitation. Insecticides, fly traps, etc. should be used as control measures against these houseflies.

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## Mathematics for Biological Sciences

By Vitthalrao B. Khyade & Hanumant V. Wanve

*Abstract-* Mathematical science and Biological sciences are interdisciplinary approaches in the field of scientific research. Both of them deserve a wide range of applications. The study of mathematics for biology is sometimes called mathematical biology or biomathematics to stress the mathematical side, or theoretical biology to stress the biological side. One can derive the quantitative genetics through consideration of infinitesimal effects at a large number of gene loci, together with the assumption of linkage equilibrium or quasi-linkage equilibrium. Ronald Fisher made The intensive work on fundamental advances in statistics (Example: Analysis of Variance) belong to Ronald Fisher. This achievement by Ronald Fisher was through his work on quantitative genetics. The phylogenetics is one more important branch of population genetics that led to the extensive development of Biological sciences through Mathematics.

*Keywords:* quantitative genetics; population dynamics; supercomplex mechanisms.

*GJSFR-C Classification:* FOR Code: 279999



*Strictly as per the compliance and regulations of:*



# Mathematics for Biological Sciences

Vitthalrao B. Khyade <sup>α</sup> & Hanumant V. Wanve <sup>ο</sup>

**Abstract-** Mathematical science and Biological sciences are interdisciplinary approaches in the field of scientific research. Both of them deserve a wide range of applications. The study of mathematics for biology is sometimes called mathematical biology or biomathematics to stress the mathematical side, or theoretical biology to stress the biological side. One can derive the quantitative genetics through consideration of infinitesimal effects at a large number of gene loci, together with the assumption of linkage equilibrium or quasi-linkage equilibrium. Ronald Fisher made the intensive work on fundamental advances in statistics (Example: Analysis of Variance) belong to Ronald Fisher. This achievement by Ronald Fisher was through his work on quantitative genetics. The phylogenetics is one more important branch of population genetics that led to the extensive development of Biological sciences through Mathematics. The Phylogenetics is the branch dealing with the reconstruction and analysis of phylogenetic (evolutionary) trees and network based on inherited characteristics. Assumptions on the "Constant Population Size" belongs to many "Population Genetics" models. The population dynamics is treating the "Variable Population Size" as absence of genetic variation. History of such type of work goes back to the 19<sup>th</sup> century. Even as far as 1798. In 1798, Thomas Malthus formulated the first principle of population dynamics. This principle later became popularize as the "Malthusian Growth Model". Alfred J. Lotka, in 1910 proposed the model of autocatalytic chemical reactions. Vito Volterra tried his best to extend this work and titled as "Lotka - Volterra Predator-Prey Equations". Basically, Vito Volterra was Mathematician. The mathematical epidemiology is the study of infectious disease affecting populations. Upto some extent, the "Population dynamics" use to overlaps mathematical epidemiology. The mathematics and Biology, both are serving a lot to orchestrate the progression of the global research.

**Keywords:** quantitative genetics; population dynamics; supercomplex mechanisms.

## I. INTRODUCTION

Mathematical and theoretical biology are the interdisciplinary scientific research fields with a range of their applications. The branch is sometimes called "Mathematical Biology" or "Biomathematics" to stress the mathematical side. It may also called as "Theoretical Biology" to stress the biological side. The "Theoretical Biology" use to focus more on the development of theoretical principles for biology. "Mathematical Biology" focuses on the use of

mathematical tools to study biological systems. According to Longo and Soto (2016) and Montévil, *et al* (2016), even though the two terms "Mathematical Biology" and "Theoretical Biology" are sometimes interchanged, the concept remains one and the same. The sole aim of "Mathematical Biology" is mathematical representation, treatment and modeling process expected in Biology. This is achieved through using techniques and tools of applied mathematics. "Mathematical Biology" has both theoretical and practical applications in research in the fields of biology, biomedical science and biotechnology. The biology use to explain the process of digestion in larval instars of silkworm, *Bombyx mori* (L) for example. The "Mathematical Biology" is expecting amount of food consumption and utilization by the tissue. "Mathematical Biology" explain in a quantitative manner, with appropriate signs and measurements (with meaningful correlation). Therefore, the biological systems can be better simulated, and hence properties can be predicted that might not be evident to the experimenter. According to Robeva, Raina (2010), Mathematical Biology employs many components of mathematics and has contributed to the development of new techniques.

There is application of Mathematics in Biology since the 19<sup>th</sup> century. Fritz Müller (Birth: 31 March 1821 – Death: 21 May 1897) (Original name: Johann Friedrich Theodor Müller) described the evolutionary benefits of adaptation of organism to environment. The adaptation of organism to environment was popularized as "Müllerian mimicry" in 1879. The account of "Müllerian mimicry" is notable for being the first use of a mathematical argument in evolutionary ecology. It explain "How powerful the effect of natural selection would be". It also highlights on "effects of population growth" The Mathematical Biology argued that population growth would be "geometric" while resources (the environment's carrying capacity) could only grow arithmetically (Mallet James, 2001).

One founding text is considered to be "On Growth and Form" (1917) by D'Arcy Thompson (Ian Stewart, 1998), and other early pioneers include Ronald Fisher, Hans Leo Przibram, Nicolas Rashevsky and Vito Volterra (Evelyn Fox Keller, 2002).

1960 is the year which shows rapid growth of the "Interest in Bio-mathematics".

Followings are some possible reasons for rapid growth of the "Interest in Bio-mathematics":

- The genomics, the newly launched area made rich collection of the data in the form of "Information".

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The genomics revolution is difficult to understand without the use of mathematical analytical tools.

- The chaos theory and the other recent development of tools in mathematical analysis to help to learn complex, non-linear mechanisms in biology.
- The calculations and the simulations were not possible before the computer era. The computers made increase in computing power. The computers allowed calculations and simulations not previously possible.
- An increasing interest in in silico experimentation due to ethical considerations, risk, unreliability and other complications involved in human and animal research.

## II. RESEARCH AREAS IN BIOMATHEMATICS

There are several areas of specialized research in mathematical and theoretical biology (Baianu, *et al*, 2006). In addition, there are external links to related projects in various universities are concisely presented in the following subsections, including also a large number of appropriate validating references from a list of several thousands of published authors contributing to this field. mechanisms. The results of such and other interactions may only be understood through a combination of mathematical, logical, physical, chemical, molecular and computational models. Many of the included parameteres are characterised by highly complex, nonlinear, and supercomplex. Generally, the biomathematical research is carried out through collaboration between mathematicians, biomathematicians, theoretical biologists, bioinformaticians, biostatisticians, physicists, biophysicists, biochemists, bioengineers, engineers, biologists, physiologists, research physicians, biomedical researchers, oncologists, molecular biologists, geneticists, embryologists, zoologists, chemists, etc. This is because of wide diversity of specific knowledge involvement.

## III. EVOLUTIONARY BIOLOGY

Traditionally, the dominant areas of mathematical biology are the ecology and evolutionary biology. There is extensive mathematical theorizing in evolutionary biology. The traditional approach with reference to population genetics is supposed to be complications from genetics in this area. In the population geneticists, it is considered that, "The appearance of new alleles by mutation, the appearance of new genotypes by recombination, and changes in the frequencies of existing alleles and genotypes at a small number of gene loci". When infinitesimal influences at a large number of gene loci are considered, together with the assumption of linkage equilibrium or quasi-linkage equilibrium, one derives quantitative genetics, the mathematical vision for biology. According to Ronald Fisher(1928), fundamental advances in statistics

correlates the mathematics and biology through the study areas such as analysis of variance, via his work on quantitative genetics. Phylogenetics is another important branch of population genetics. It lead to the extensive development of coalescent theory is phylogenetics. Phylogenetics is dealing with the reconstruction and analysis of phylogenetic (or evolutionary) trees and networks based on inherited characteristics (Charles Semple, 2003). Traditional population genetic models is dealing with alleles and genotypes, and are frequently stochastic.

According to population genetics models, population sizes are constant. The variable sizes of the population, often in the absence of genetic variation, are treated by the field of population dynamics. The history of work in this area goes back to the 19<sup>th</sup> century. The supportive documents are even as far as 1798 when Thomas Malthus formulated the first principle of population dynamics. Later, the formulation of Thomas Malthus became known as the "Malthusian Growth Model". The Lotka–Volterra predator-prey equations are another famous example. Population dynamics overlap with another active area of research in mathematical biology: mathematical epidemiology, the study of infectious disease affecting populations. Various models of the spread of infections have been proposed and analyzed, and provide important results that may be applied to health policy decisions.

John Maynard Smith and George R. Price developed the evolutionary game theory. According to this theory, the "Selection acts directly on inherited phenotypes, without genetic complications". Further, this approach has been mathematically reshuffled to produce the field of adaptive dynamics.

## IV. THEORY OF "COMPUTER MODELS AND AUTOMATA"

A document on the "Theory of Computer Models and Automata" summarizes an extensive amount of published research in this area up to 1986 (Witten, 1986; Lin, 2004). This attempt tried it's best to include subsections in the following areas: The computer modeling in biology and medicine; The arterial system models; the neuron models; the biochemical and oscillation networks; the quantum automata; the quantum computers in molecular biology and genetics; the cancer modeling (Baianu, 2004); the neural nets; the genetic networks; the abstract categories in relational biology (Kainen, 2005); the metabolic-replication systems; the category theory applications in biology and medicine; the automata theory; the cellular automata; the tessellation models and complete self-reproduction; the chaotic systems in organisms; the relational biology and organismic theories (Baianu,1987). The growing importance of molecular biology boosted area of "cell

and molecular biology". Mechanics of biological tissues (Ray Ogden, 2004).

- Theoretical enzymology and enzyme kinetics
- Cancer modelling and simulation Oprisan, *et al*, 2006)
- Modelling the movement of interacting cell populations (Wolkenhauer, 2004)
- Mathematical modelling of scar tissue formation
- Mathematical modelling of intracellular dynamics (Kuznetsov and Avramenko, 2009).
- Mathematical modelling of the cell cycle (Noe, *et al*, 2017)
- Modelling of arterial diseases cycle (Noe, *et al*, 2017)
- Multi-scale modelling of the heart cycle (Noe, *et al*, 2017)
- Modelling electrical properties of muscle interactions, as in bidomain and monodomain models.

## V. MOLECULAR SET THEORY: MATHEMATICAL FORMULATION FOR BIOCHEMICAL KINETICS

The Molecular Set Theory (MST) was introduced by Anthony Bartholomay. It is a mathematical formulation of the wide-sense chemical kinetics of biomolecular reactions in terms of sets of molecules and their chemical transformations represented by set-theoretical mappings between molecular sets. The applications of Molecular Set Theory (MST) were developed in mathematical biology and especially in mathematical medicine. In a more general sense, MST is the theory of molecular categories defined as categories of molecular sets and their chemical transformations represented as set-theoretical mappings of molecular sets. It has also contributed to biostatistics and the formulation of clinical biochemistry problems in mathematical formulations of pathological changes, biochemical changes of interest to Physiology, changes in Clinical Biochemistry and Medicine (Noe, *et al* 2017). The term "Model" is often used synonymously with the "Corresponding System of Equations". In the Molecular Set Theory (MST), there is conversion of model of a biological system into a system of equations. This system of equation use to help to solve and find the solution of the equations. This is employed by either analytical or numerical means. Each and every step in the solution explains "How the biological system behaves either over time or at equilibrium". Different types of behavior or the factors of biological systems affect on the corresponding system of equations. There are many different types of equations and the type of behavior that can occur is dependent on both the model and the equations used. The model often makes assumptions about the system. The equations may also

make assumptions about the nature of what may occur (Noe, *et al*, 2017).

Due to the recent significant modifications in performance, the computer accelerates the model simulation based on various formulas. The Bio-Math-Modeler websites can carry out simulations and display charts interactively on browser. The earlier phases of mathematical-biology was dominated by mathematical-biophysics. The mathematical-biophysics described as the application of mathematics in biophysics. It is generally, involving specific physical or mathematical models of systems, components or compartments in biosystems.

## VI. BIOLOGICAL ORGANIZATION

The aim of theoretical approaches to biological organization is to understand the interdependence between the parts in the body of organisms. These approaches emphasize the circularities that these interdependences lead to. Theoretical biologists developed several concepts to formalize the concept of "Interdependence of various organs in the body". Abstract Relation Biology (ARB), for example, is concerned with the study of general, relational models of complex biological systems. The ARB, usually abstracting out specific morphological, or anatomical, structures. Some of the simplest models in ARB are the Metabolic-Replication (MR) and Relational models. The Metabolic-Replication (M R) was the systems introduced by Robert Rosen in 1957-1958 as abstract. The relational models was introduced by Rosen Robert in 2005. The relational model was to introduce the concept of cellular and organismal organizations. The other theoretical approaches to biological organization include the notion of autopoiesis developed by Maturana and Varela, Kauffman's Work-Constraints cycles, and more recently the notion of closure of constraints (.....reference.....?.....).

## VII. ALGEBRAIC BIOLOGY

The algebraic biology is also known as "Symbolic-Systems-Biology". It applies the algebraic methods of symbolic computation to the study of problems in biology, especially in genomics, proteomics, analysis of molecular structures and study of genes (Michael P Barnett, 2005).

## VIII. THE CELL CYCLE: IDEAL EXAMPLE MODEL EXAMPLE FOR BIOMATHEMATICS

Sequential and repetitive events in the life of the cell may be called as cell cycle. It is the series of events that take place in a cell leading to its division and duplication through its DNA (DNA replication) to produce two daughter cells. In prokaryotes (ex. bacteria), there is no nuclear membrane to keep the nuclear material separate from cytoplasm. That is to say

the nuclear material and cytoplasmic material are mixed with each other. The cell cycle in prokaryotes is divided into the periods, may be entitled: Period: "B", Period: "C", and Period: "D" periods. The Period: "B" extend from the end of cell division to the beginning of replication of DNA. The replication of DNA occurs during the Period: "C". The Period: "D" refers to the stage between the end of the replication of DNA and the splitting of the bacterial cell into two daughter cells (Michael P Barnett, 2005). In eukaryotes, the cell is with a nucleus. The eukaryotic cell cycle is divided into three periods or phases: interphase (I), the mitotic (M) phase and cytokinesis (C). During interphase (I), there is growth of cell. The cell is accumulating nutrients needed for the cell division. There is preparation of cell itself for cell-division and duplicating its DNA. During the mitotic (M) phase, the chromosomes duplicate and separate. During the final stage, cytokinesis (C), the chromosomes separate followed by cytoplasmic division yielding the daughter cells. To ensure the proper division of the cell, there are control mechanisms known as cell cycle checkpoints.

The cell-division cycle deserve vital virtualness. The cell division is the process by which a single-celled fertilized egg (Zygote) develops into a mature organism. The cell division is required for the growth and life of organs in multicellular organisms. There is renewal of many parts of the body through the process of cell division. Soon after cell division, the daughter cells enters in the interphase of a new cell cycle. Each phase of the cell cycle significant set of specialized biochemical process. The misregulation of cell cycle in eukaryotic organisms may leads to cancers. Most possibly, the cell cycle is a good example of a mathematical model. It deals with simple calculus but gives valid results. Two research groups. The "Generic Eukaryotic Cell Cycle Model" is representing a particular eukaryote depending on the values of the parameters. It is demonstrating the "Idiosyncrasies" of the individual cell cycle. Idiosyncrasies are formed through to different protein concentrations and affinities. The underlying mechanisms of diosyncrasies formation are conserved (Csikasz-Nagy, *et al.*, 2006).

In deterministic process, models show the change in time (dynamical system) of the protein inside a single typical cell. In stochastic process, the model is describing a statistical distribution of protein concentrations in a population of cells is called a stochastic process.

For the purpose to get mathematical equations, an iterative series of steps include:

- (I) The observations of several models.
- (II) Formation of Consensus Diagram based on observations of several models.
- (III) Selection of appropriate kinetic Laws or the principles.

- (IV) Establishment of Mathematical Equation (may be in the form of differential equation). For example: The rate kinetics for stoichiometric reactions, Michaelis-Menten kinetics for enzyme substrate reactions and Goldbeter-Koshland kinetics for ultrasensitive transcription factors.
- (V) Fitting the parameters of the equations (rate constants, enzyme efficiency coefficients and Michaelis constants). This is to match observations. When they cannot be fitted, the kinetic equation is revised and when that is not possible the wiring diagram is modified.
- (VI) Validation of the equation using observations of both, in laboratory readings and field readings.

For the purpose to fit the parameters, the differential equations must be studied. This can be done either through simulation or through analysis. In a simulation, there is calculation of progression of the system through the use of given a starting vector (list of the values of the variables). It is followed by solving the equations at each time-frame in small increments.

In the analysis, there is investigation of the behavior of system (depending on the values of the parameters and the variables) through the use of properties of the equations. A system of differential equations for example, can be represented as a vector field, where each vector described the change (in concentration of two or more protein) determining where and how fast the trajectory (simulation) is heading. The fields of Vector can have several special points, which include: a stable point, called a sink, that attracts in all directions (forcing the concentrations to be at a certain value), an unstable point, either a source or a saddle point, which repels (forcing the concentrations to change away from a certain value), and a limit cycle, a closed trajectory towards which several trajectories spiral towards (making the concentrations oscillate).

Through the use of bifurcation theory, it is possible for better representation of the biological data. It handles the large number of variables and parameters. In a biochemical reaction, there is a special steady-state points at certain values of a parameter. These parameters are represented by a point. Once the parameter passes a certain value, it yield a qualitative change. This qualitative change, at certain point of known variable (or parameter) is nothing but a bifurcation. In a cell cycle, nature of the space changes, with profound consequences for the protein concentrations. In terms of mathematics, cell cycle has phases like G.1 and G.2. The level of cyclin in each of the phase controls the process of cell cycle. Through the stable point, mass of the cell, controls cyclin levels. In the Synthetic phase and M phase, the concentrations change independently. It is impossible for the system to go back to the previous level. This because, at the current mass the vector field is profoundly different and

the mass cannot be reversed back through the bifurcation event. The event of bifurcation is responsible for making a checkpoint irreversible. In particular, in S phase and M phase, the checkpoints are regulated by means of special bifurcations called a Hopf bifurcation and an infinite period bifurcation.

## IX. CONCLUSION

The study of mathematics for biology is sometimes called mathematical biology or biomathematics to stress the mathematical side, or theoretical biology to stress the biological side. One can derive the quantitative genetics through consideration of infinitesimal effects at a large number of gene loci, together with the assumption of linkage equilibrium or quasi-linkage equilibrium. Ronald Fisher made the intensive work on fundamental advances in statistics (Example: Analysis of Variance) belong to Ronald Fisher. This achievement by Ronald Fisher was through his work on quantitative genetics. The phylogenetics is one more important branch of population genetics that led to the extensive development of Biological sciences through Mathematics. The Phylogenetics is the branch dealing with the reconstruction and analysis of phylogenetic (evolutionary) trees and network based on inherited characteristics. Assumptions on the "Constant Population Size" belongs to many "Population Genetics" models. The population dynamics is treating the "Variable Population Size" as absence of genetic variation. History of such type of work goes back to the 19<sup>th</sup> century. Even as far as 1798. In 1798, Thomas Malthus formulated the first principle of population dynamics. This principle later became popularize as the "Malthusian Growth Model". Alfred J. Lotka, in 1910 proposed the model of autocatalytic chemical reactions. Vito Volterra tried his best to extend this work and titled as "Lotka - Volterra Predator-Prey Equations". Basically, Vito Volterra was Mathematician. The mathematical epidemiology is the study of infectious disease affecting populations. Upto some extent, the "Population dynamics" use to overlaps mathematical epidemiology.

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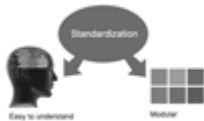
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**Note :**

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- In future, if the board feels the necessity to change any board member, the same can be done with the consent of the chairperson along with anyone board member without our approval.
- In case, the chairperson needs to be replaced then consent of 2/3rd board members are required and they are also required to jointly pass the resolution copy of which should be sent to us. In such case, it will be compulsory to obtain our approval before replacement.
- In case of “Difference of Opinion [if any]” among the Board members, our decision will be final and binding to everyone.

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# PREFERRED AUTHOR GUIDELINES

**We accept the manuscript submissions in any standard (generic) format.**

We typeset manuscripts using advanced typesetting tools like Adobe In Design, CorelDraw, TeXnicCenter, and TeXStudio. We usually recommend authors submit their research using any standard format they are comfortable with, and let Global Journals do the rest.

Alternatively, you can download our basic template from <https://globaljournals.org/Template.zip>

Authors should submit their complete paper/article, including text illustrations, graphics, conclusions, artwork, and tables. Authors who are not able to submit manuscript using the form above can email the manuscript department at [submit@globaljournals.org](mailto:submit@globaljournals.org) or get in touch with [chiefeditor@globaljournals.org](mailto:chiefeditor@globaljournals.org) if they wish to send the abstract before submission.

## BEFORE AND DURING SUBMISSION

Authors must ensure the information provided during the submission of a paper is authentic. Please go through the following checklist before submitting:

1. Authors must go through the complete author guideline and understand and *agree to Global Journals' ethics and code of conduct*, along with author responsibilities.
2. Authors must accept the privacy policy, terms, and conditions of Global Journals.
3. Ensure corresponding author's email address and postal address are accurate and reachable.
4. Manuscript to be submitted must include keywords, an abstract, a paper title, co-author(s) names and details (email address, name, phone number, and institution), figures and illustrations in vector format including appropriate captions, tables, including titles and footnotes, a conclusion, results, acknowledgments and references.
5. Authors should submit paper in a ZIP archive if any supplementary files are required along with the paper.
6. Proper permissions must be acquired for the use of any copyrighted material.
7. Manuscript submitted *must not have been submitted or published elsewhere* and all authors must be aware of the submission.

## Declaration of Conflicts of Interest

It is required for authors to declare all financial, institutional, and personal relationships with other individuals and organizations that could influence (bias) their research.

## POLICY ON PLAGIARISM

Plagiarism is not acceptable in Global Journals submissions at all.

Plagiarized content will not be considered for publication. We reserve the right to inform authors' institutions about plagiarism detected either before or after publication. If plagiarism is identified, we will follow COPE guidelines:

Authors are solely responsible for all the plagiarism that is found. The author must not fabricate, falsify or plagiarize existing research data. The following, if copied, will be considered plagiarism:

- Words (language)
- Ideas
- Findings
- Writings
- Diagrams
- Graphs
- Illustrations
- Lectures



- Printed material
- Graphic representations
- Computer programs
- Electronic material
- Any other original work

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1. Substantial contributions to the conception and acquisition of data, analysis, and interpretation of findings.
2. Drafting the paper and revising it critically regarding important academic content.
3. Final approval of the version of the paper to be published.

### Changes in Authorship

The corresponding author should mention the name and complete details of all co-authors during submission and in manuscript. We support addition, rearrangement, manipulation, and deletions in authors list till the early view publication of the journal. We expect that corresponding author will notify all co-authors of submission. We follow COPE guidelines for changes in authorship.

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### Appealing Decisions

Unless specified in the notification, the Editorial Board's decision on publication of the paper is final and cannot be appealed before making the major change in the manuscript.

### Acknowledgments

Contributors to the research other than authors credited should be mentioned in Acknowledgments. The source of funding for the research can be included. Suppliers of resources may be mentioned along with their addresses.

### Declaration of funding sources

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## PREPARING YOUR MANUSCRIPT

Authors can submit papers and articles in an acceptable file format: MS Word (doc, docx), LaTeX (.tex, .zip or .rar including all of your files), Adobe PDF (.pdf), rich text format (.rtf), simple text document (.txt), Open Document Text (.odt), and Apple Pages (.pages). Our professional layout editors will format the entire paper according to our official guidelines. This is one of the highlights of publishing with Global Journals—authors should not be concerned about the formatting of their paper. Global Journals accepts articles and manuscripts in every major language, be it Spanish, Chinese, Japanese, Portuguese, Russian, French, German, Dutch, Italian, Greek, or any other national language, but the title, subtitle, and abstract should be in English. This will facilitate indexing and the pre-peer review process.

The following is the official style and template developed for publication of a research paper. Authors are not required to follow this style during the submission of the paper. It is just for reference purposes.



### ***Manuscript Style Instruction (Optional)***

- Microsoft Word Document Setting Instructions.
- Font type of all text should be Swis721 Lt BT.
- Page size: 8.27" x 11", left margin: 0.65, right margin: 0.65, bottom margin: 0.75.
- Paper title should be in one column of font size 24.
- Author name in font size of 11 in one column.
- Abstract: font size 9 with the word "Abstract" in bold italics.
- Main text: font size 10 with two justified columns.
- Two columns with equal column width of 3.38 and spacing of 0.2.
- First character must be three lines drop-capped.
- The paragraph before spacing of 1 pt and after of 0 pt.
- Line spacing of 1 pt.
- Large images must be in one column.
- The names of first main headings (Heading 1) must be in Roman font, capital letters, and font size of 10.
- The names of second main headings (Heading 2) must not include numbers and must be in italics with a font size of 10.

### ***Structure and Format of Manuscript***

The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

A research paper must include:

- a) A title which should be relevant to the theme of the paper.
- b) A summary, known as an abstract (less than 150 words), containing the major results and conclusions.
- c) Up to 10 keywords that precisely identify the paper's subject, purpose, and focus.
- d) An introduction, giving fundamental background objectives.
- e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition, sources of information must be given, and numerical methods must be specified by reference.
- f) Results which should be presented concisely by well-designed tables and figures.
- g) Suitable statistical data should also be given.
- h) All data must have been gathered with attention to numerical detail in the planning stage.

Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

- i) Discussion should cover implications and consequences and not just recapitulate the results; conclusions should also be summarized.
- j) There should be brief acknowledgments.
- k) There ought to be references in the conventional format. Global Journals recommends APA format.

Authors should carefully consider the preparation of papers to ensure that they communicate effectively. Papers are much more likely to be accepted if they are carefully designed and laid out, contain few or no errors, are summarizing, and follow instructions. They will also be published with much fewer delays than those that require much technical and editorial correction.

The Editorial Board reserves the right to make literary corrections and suggestions to improve brevity.



## FORMAT STRUCTURE

***It is necessary that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.***

All manuscripts submitted to Global Journals should include:

### **Title**

The title page must carry an informative title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) where the work was carried out.

### **Author details**

The full postal address of any related author(s) must be specified.

### **Abstract**

The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

### **Keywords**

A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy: planning of a list of possible keywords and phrases to try.

Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning searching. Think about the most important concepts related to research work. Ask, "What words would a source have to include to be truly valuable in a research paper?" Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

### **Numerical Methods**

Numerical methods used should be transparent and, where appropriate, supported by references.

### **Abbreviations**

Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

### **Formulas and equations**

Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

### **Tables, Figures, and Figure Legends**

Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.



## Figures

Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

## PREPARATION OF ELETRONIC FIGURES FOR PUBLICATION

Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/ photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). Please give the data for figures in black and white or submit a Color Work Agreement form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution at final image size ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs): >350 dpi; figures containing both halftone and line images: >650 dpi.

Color charges: Authors are advised to pay the full cost for the reproduction of their color artwork. Hence, please note that if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a Color Work Agreement form before your paper can be published. Also, you can email your editor to remove the color fee after acceptance of the paper.

## TIPS FOR WRITING A GOOD QUALITY SCIENCE FRONTIER RESEARCH PAPER

Techniques for writing a good quality Science Frontier Research paper:

**1. Choosing the topic:** In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

**2. Think like evaluators:** If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

**3. Ask your guides:** If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

**4. Use of computer is recommended:** As you are doing research in the field of science frontier then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

**5. Use the internet for help:** An excellent start for your paper is using Google. It is a wondrous search engine, where you can have your doubts resolved. You may also read some answers for the frequent question of how to write your research paper or find a model research paper. You can download books from the internet. If you have all the required books, place importance on reading, selecting, and analyzing the specified information. Then sketch out your research paper. Use big pictures: You may use encyclopedias like Wikipedia to get pictures with the best resolution. At Global Journals, you should strictly follow here.



**6. Bookmarks are useful:** When you read any book or magazine, you generally use bookmarks, right? It is a good habit which helps to not lose your continuity. You should always use bookmarks while searching on the internet also, which will make your search easier.

**7. Revise what you wrote:** When you write anything, always read it, summarize it, and then finalize it.

**8. Make every effort:** Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

**9. Produce good diagrams of your own:** Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

**10. Use proper verb tense:** Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

**11. Pick a good study spot:** Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

**12. Know what you know:** Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

**13. Use good grammar:** Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

**14. Arrangement of information:** Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

**15. Never start at the last minute:** Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

**16. Multitasking in research is not good:** Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

**17. Never copy others' work:** Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

**18. Go to seminars:** Attend seminars if the topic is relevant to your research area. Utilize all your resources.

**19. Refresh your mind after intervals:** Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.



**20. Think technically:** Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

**21. Adding unnecessary information:** Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

**22. Report concluded results:** Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

**23. Upon conclusion:** Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium through which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

## INFORMAL GUIDELINES OF RESEARCH PAPER WRITING

### **Key points to remember:**

- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

### **Final points:**

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

*The introduction:* This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

### **The discussion section:**

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

### **General style:**

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

**To make a paper clear:** Adhere to recommended page limits.



### *Mistakes to avoid:*

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

### **Title page:**

Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

**Abstract:** This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

*Reason for writing the article—theory, overall issue, purpose.*

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

### **Approach:**

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

### **Introduction:**

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.



*The following approach can create a valuable beginning:*

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

#### **Approach:**

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

#### **Procedures (methods and materials):**

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

#### **Materials:**

*Materials may be reported in part of a section or else they may be recognized along with your measures.*

#### **Methods:**

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

#### **Approach:**

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

#### **What to keep away from:**

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.



**Results:**

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

**Content:**

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

**What to stay away from:**

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

**Approach:**

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

**Figures and tables:**

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

**Discussion:**

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."



Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

**Approach:**

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