BIOLOGICAL SCIENCES DIVISION

CHEMICAL PROTECTION FROM RADIATION-INDUCED GASTROINTESTINAL SYNDROME USING RADIOPROTECTORS FROM HERBAL SOURCES, GC-2112 AND GX-2137

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ABSTRACT

A wide range of clinical manifestations of tissue morbidities belonging to the Acute Radiation Complex (ARC) are the major limiting factors which radiation oncologists are primarily concerned with during clinical radio therapy of tumors. Among such critical events is the development of the gastrointestinal (GI) syndrome correlated to the degeneration of the intestinal mucosa following irradiation at absorbed doses of least 15-20 Gy. The possibility of using herbal products, GX-2137 from ginseng (*Panax* sp.), and GC-2112 from garlic *(Allium sativum),* in conferring protection of the duodenum was investigated. The post-irradiation response kinetics of critical tissue parameters (length of villi, number of crypts and villi cells) are analyzed in ICR mice exposed to γ-radiation at absorbed doses: 1.5, 5, 20, 5 Gy using *in situ* microcolony survival and apoptosis assays. Individual crypts reveal time-dependent, differential modalities of radiation death governed by a single-hit target inactivation at 2 h and a multi-hit, multi-target-inactivation phenomenon at

48 h post-irradiation. The behaviors of cryptogenic survival are altered by GC-2112 which are apparent from the increased total crypt cell count at 2 h and the extension of the shoulder region of the biphasic survival curve after 48 h. In addition, a general reduction in apoptatic indices is shown in GC-212-protected duodenum by shifting the single-hit target survival curve, characteristic of apoptatic induction phenomena, to a multi-hit, target-inactivation scheme. Using the linear quadratic (LQ) biomechanistic analysis, utilized in tumor-radioresponse modeling studies, to determine possible mechanisms of action by GC-2112, results suggest that this herbal product protects the crypt cells from apoptotic and clonogenic deaths by preventing β-lethality marked by a decrease in the amount of accumulated radiation-induced sublethal damages without altering intrinsic radiosensitivities of the crypts. In contrast, radioprotection by GX-2137 was not detected. On the other hand, damage to the villi structures, a more radioresistant parameter, is significantly minimized using both radioprotectors at the given radiation doses.

CARBONIC ANHYDRASE: ITS PHYSIOLOGICAL AND EVOLUTIONARY SIGNIFICANCE IN THE MARINE SYMBIONT *Prochloron*

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ABSTRACT

The activity of carbonic anhydrase (CA), a photosynthetic enzyme catalyzing the reversible interconversion of HCO_3^- to CO_2, was studied in *Prochloron*. Measurements revealed that this prokaryotic microalgal symbiont of tropical ascidians exhibits CA activity largely associated with the cell surface. Similar to some chlorophytes, the predominance of extracellular CA and its inhibition increased the $K_{1/2}$ ($NaHCO_3$) for photosynthesis suggesting that extracellular CA in *Prochloron* is important in facilitating the supply of CO_2 into the cell from HCO_3^- which is the form common at high pH values, such as in seawater.

Examination of the effect of sulfonamide inhibitors, acetazolamide and ethoxzolamide, revealed that CA activity of *Prochloron* is inhibited with I_{50} values of 700 μ*M* and 300 μ*M*, respectively. These I_{50} values bear close resemblance to the measured I_{50} values for unicellular cyanobacteria and chloroplasts of green algae and higher plants. Since *Prochloron* shares characters with both cyanobacteria and green chloroplasts, it could then be placed as the possible evolutionary link between the cyanobacteria and chlorophytes.

INTRODUCTION

The genus *Prochloron* consists of marine unicellular algae found in symbiotic association with certain tropical didemnid ascidians. They occur in intimate but extracellular association with the ascidian host colony, either attached to the outer surface, embedded in the test or lying in the common cloacal cavity [12]. Earlier studies showed that they are prokaryotic, with an ultrastructure resembling that of cyanobacteria or blue-green algae [26]. However, they apparently lack the distinctive photosynthetic pigments, phycobilins, of the cyanobacteria, contain both chlorophylls a and b, and have paired or stacked thylakoids like those of eukaryotic chlorophytes or green algae [27]. Their discovery has generated considerable

excitement in the scientific community due to its bearing on theories of the origin of eukaryotic chloroplasts, and has prompted much speculation with regard to their unique position in algal phylogeny.

Measurements of photosynthesis revealed the operation of the C_3 photosynthetic pathway in *Prochloron* with 3-phosphoglycerate as the first carbon fixation product [2] and ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO) as the primary carboxylation enzyme [8]. Since CO_2 is the primary substrate for caboxylation by RuBisCO, and not HCO_3^-, the commonest form of inorganic carbon in seawater, the enzyme carbonic anhydrase (CA) which catalyzes the conversion of HCO_3^- to CO_2, was previously assumed to be present in these organisms [3].

In this paper, the actual presence of carbonic anhydrase in *Prochloron* will be shown and the physiological and evolutionary significance of this enzyme in this particular microalga will be analyzed.

MATERIALS AND METHODS

Collection of ascidian colony and isolation of *Prochloron* cells

Colonies of the ascidian host, *Lissoclinum bistratum* growing on patches of benthic macrophytes and on the leaves of seagrasses or *L. patella* growing on the upper surfaces of coral rubble were collected at Palau, West Caroline Islands. The animal colonies, usually found 1-3 m below surface water, were taken and promptly transported in seawater to the laboratory aboard the Japanese research vessel Sohgen-Maru. Individual colonies were cleaned of contaminants and the algal cells isolated from the host by squeezing gently by hand. The algae were then received in seawater buffered with 40 m*M* Tris at pH 8.4 and concentrated by centrifugation at about 60 x g for 120 sec.

Measurement of carbonic anhydrase activity

For the assay of CA activity, the algae isolated from the host were suspended in 20 m*M* Veronal-H_2SO_4 buffer pH 8.3. The enzyme activity on the cell surface (extracellular activity) was assayed directly on such suspensions whereas total activity was assayed in homogenates disrupted by sonication. The difference between the total and extracellular activities represents the intracellular activity. When the effects of CA inhibitors, acetazolamide (AZA) and ethoxzolamide (EZA) were examined, small volumes of the compounds were added to the assay buffer prior to addition of the sample, to provide the appropriate final concentration. The assay method and expression of CA activity were the same as described previously[14] the enzyme activity units expressed on a chlorophyll basis. The concentration of chlorophyll extracted with methanol was determined according to Mackinney[28].

Determination of photosynthetic oxygen evolution

Cells collected by centrifugation were washed twice and suspended in freshly prepared CO_2-free seawater buffered with 40 m*M* Tris at pH 8.4. The cell suspension (5 mL) at a density of 10 mg chlorophyll per liter was placed in a water-jacketed cylinder equipped with a Clark-type oxygen probe. This was illuminated from one side by a projector lamp at the desired photon flux density of 250 μmol m^{-2} sec^{-1}. The temperature was kept at 30°C by water running through the water jacket and a thermostat. Initially, the algal suspension was preilluminated until the endogenous carbon source was depleted as measured by cessation of oxygen evolution. The photosynthetic reaction was then started by injecting known amounts of $NaHCO_3$ solution through narrow hole in the cap of the reaction vessel. Change of oxygen concentrations in the algal suspension was monitored with a recorder connected to the oxygen probe.

Detection of CA with antiserum

For the detection of CA protein with antiserum, electrophoresis of soluble protein extracts was first carried out on 12.5% (w/v) polyacrylamide gel according to Laemmli[24] and electrotransferred to polyvinylidene difluoride filter (Bio-Rad, Richmond, Calif., USA)[43]. The electrotransferred proteins in the filter blot were then probed with antiserum against extracellular CA of *Chlamydomonas* [15] and spinach chloroplastic CA. Bound CA antibodies in the filter were detected with goat anti-rabbit IgG conjugated horseradish peroxidase acting upon 3,3´-diaminobenzidine tetrahydrochloride[13].

RESULTS

Measurement of CA activity of *Prochloron* isolated from *Lissoclinum bistratum* and *L. patella* showed that both species exhibited activity majority (90%) of which is located on the cell surface, and only about 10% of the total CA activity is located intracellularly (Table 1). The possibility that this extracellular CA activity may be attributed to the ascidian host can be excluded since contamination by the host tissue in the cell preparation is negligible and measurement of CA activity in the animal tissue after removal of the algal cells did not show any activity.

To determine the characteristic features of CA in this microalga, the effects of the two most widely used potent sulfonamide CA inhibitors, acetazolamide (AZA) and ethoxzolamide (EZA), on CA activity of intact cells of *Prochloron* isolated from *L. patella* were examined. Sulfonamides were chosen since they were long recognized as specific high-affinity inhibitors of CA from a variety of sources [30]. The measured I_{50} values, which are the concentrations of the inhibitors required to cause 50% inhibition of activity, for the inhibition by AZA and EZA of extracellular CA from *Prochloron*, are 700 μM and 300 μM, respectively [16].

Table 1. Carbonic anhydrase activity of *Prochloron* cells isolated from their ascidian host

Ascidian host	*CA Activity (U·mg chl^{-1})*		
	Extracellular	*Intracellular*	*Total*
Lissoclinum bistratum	6.21	0.56	6.77
Lissoclinum patella	5.35	0.57	5.92

The effect of this acetazolamide concentration on the rate of photosynthetic oxygen evolution of *Prochloron* at varying $NaHCO_3$ concentrations was then studied. At the optimum photon flux density of 250 μmol m^{-2} sec^{-1}, addition of 700 μM AZA lowered the rates of photosynthesis under low $NaHCO_3$ concentrations, while it did not significantly affect the rates under saturating $NaHCO_3$ concentrations, (Fig. 1). As a consequence, the apparent affinity for inorganic carbon at low $NaHCO_3$ concentrations, measured as $K_{1/2}$ ($NaHCO_3$), at pH 8.4 increased from 160 μ*M* 230 μM by acetazolamide addition. Since AZA is a membrane-impermeable sulfonamide[35], this result indicates that CA located on the cell surface of *Prochloron* increased the affinity for CO_2 in photosynthesis at low inorganic carbon concentration.

Comparisons of the measured I_{50} values for inhibition of *Prochloron* CA by AZA and EZA with published data of CA from a variety of sources are shown in Table 2. It can be observed that the I_{50} values for *Prochloron* are very high compared to those measured for human red cell isozymes [31], the extracellular CA of unicellular chlorophyte *Chlamydomonas* [9], and the intracellular CAs of the unicellular rhodophyte *Porphyridium* [51] and filamentous cyanobacterium *Anabaena* [53]. On the other hand, the unicellular cyanobacterium *Synechococcus*[6] exhibits an I_{50} value for EZA inhibition similar to *Prochloron*. Likewise, *Prochloron* CA is similar to higher plant CA, such as spinach CA [10] and pea CA[4], and the intracellular CA of *Chlamydomonas* [21] in terms of sulfonamide inhibition.

To determine whether *Prochloron* CA is immunologically related to higher plant CA, like spinach CA, and not to *Chlamydomonas* extracellular CA as suggested from the sulfonamide inhibition results, immunoblot analysis was carried out using antisera against these two CAs (Fig. 2). The anti-extracellular CA antibody reacted with the 37 kilodalton (kDa) CA monomer in the soluble protein extracts from *Chlamydomonas*. It did not, however, cross-react with soluble protein extracts from spinach or *Prochloron*, thus confirming the inhibition results. Similarly, the anti-spinach CA antibody did not cross-react with *Chlamydomonas* CA. With *Prochloron*, however, a single immunosignal of approximately 34 kDa was observed with anti-spinach CA antibody. Since *Prochloron* CA and spinach CA exhibit almost the same sensitivity to sulfonamide, the 34 kDa band which is antigenically similar to spinach CA might be the *Prochloron* CA. This result, however, should

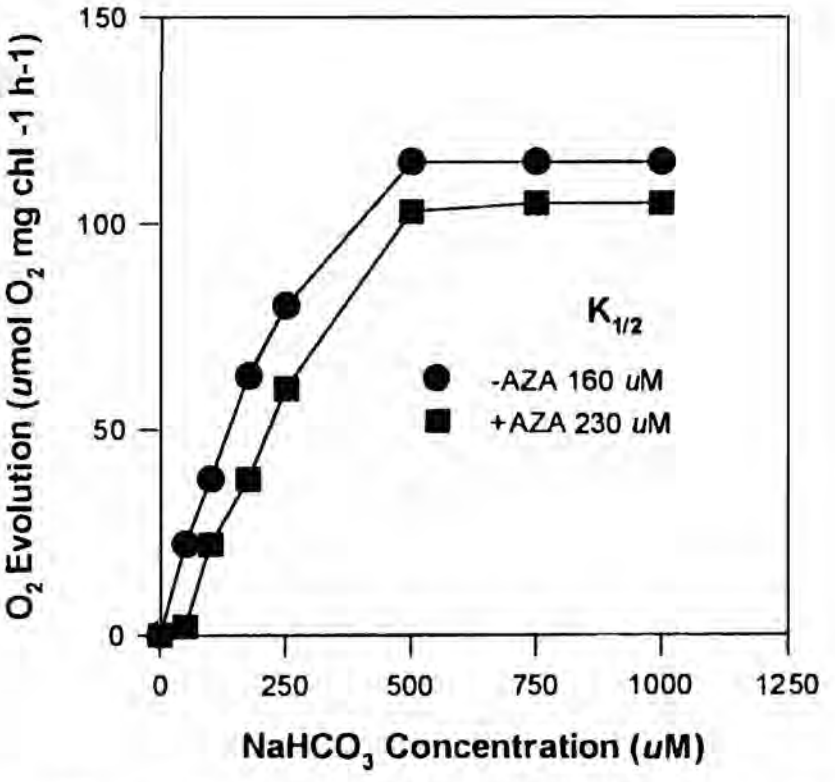

Figure 1. Effect of CA inhibitor acetazolamide on the photosynthetic rates of Prochloron at varying concentrations of $NaHCO_3$. Rate of oxygen evolution determined in the absence (•) and presence (▪) of 700 μM acetazolamide.

be taken with caution since, aside from the major 26 kDa band which corresponds to the spinach CA monomer, the antibody also reacted with other proteins in the extracts, indicating the low specificity of the antiserum used.

DISCUSSION OF RESULTS

Previous reports have shown that microalgae have CA localized either on the cell surface and/or inside the cells [1, 32, 46]. Among these microalgae, most chlorophytes exhibit CA activity which is predominantly associated with the cell surface [1]. In cyanobacteria, CA activity is localized inside the cells and no extracellular CA activity has been reported to date [6, 25, 53]. *Prochloron*, although a prokaryote, differs then from the cyanobacterial group, and bears close resemblance to chlorophytes, in exhibiting CA activity predominantly on the cell surface (Table 1).

With regards to the role of this extracellular CA in *Prochloron*, acetazolamide addition caused a decrease in the efficiency with which external inorganic carbon is used for photosynthesis (Fig. 1). This result is consistent with the suggested role of CA in various microalgae, that is, extracellular CA which is located either in the periplasmic space or attached to the cell wall [23] functions in increasing the efficiency

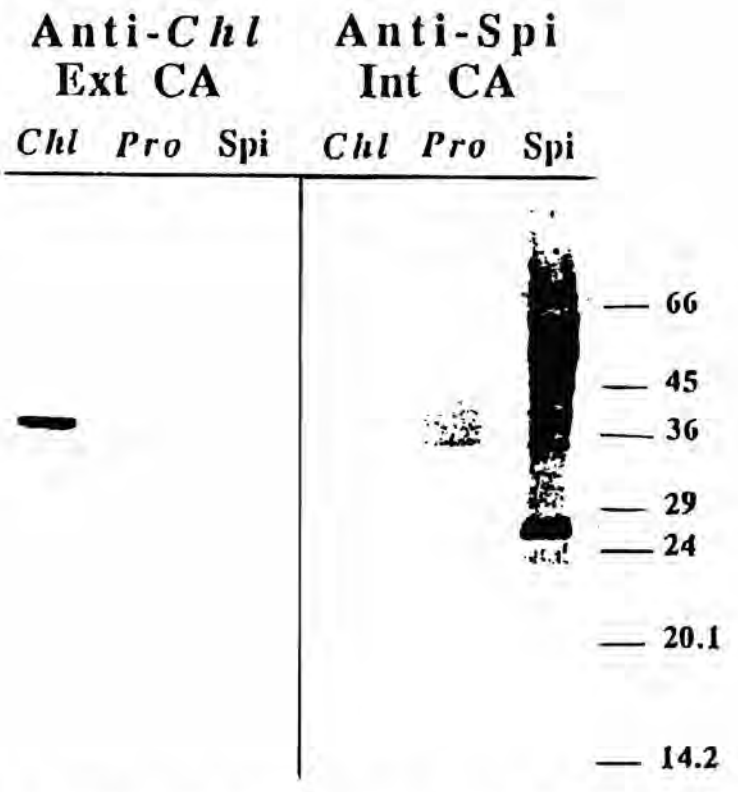

Figure 2. Immunoblots of soluble protein extracts from *Chlamydomonas (Chl), Prochloron (Pro),* and spinach (Spi) probed with antibodies against extracellular CA of *Chlamydomonas* (Anti-*Chl* Ext CA) and spinach intracellular CA (Anti-Spi Int (CA). Molecular weight markers indicated to the right in the figure are in kilodaltons.

with which cells can access external inorganic carbon [7, 41]. This involves facilitating the supply of CO_2 into the cell from HCO_3^- which is the form predominant at high pH values [32, 43]. Thus, with the aid of extracellular CA, *Prochloron* cells have access to the large HCO_3^- pool at alkaline pH values in seawater via indirect acquisition of HCO_3^-. As to the role of intracellular CA, it is thought to be involved in increasing the steady-state flux of CO_2 within the cell thereby enhancing the supply of CO_2 to RuBisCO [5, 22, 44].

Though CA in both *Synechococcus* and *Anabaena* is assumed to be localized within the RuBisCO-containing carboxysomes [6], these two cyanobacteria exhibit highly different I_{50} values in terms of CA inhibition by sulfonamides; the former is less sensitive than the latter (Table 2). It is interesting to note that on the basis of 16S ribosomal RNA sequence data, these species are two of the most highly divergent cyanobacteria known [17]. Since inhibitors like sulfonamides are thought to bind near the active site of the enzyme [31], the difference in sensitivity to sulfonamides may reflect differences at or near the active site of the these enzymes. Another microalga whose CA activity is sensitive to sulfonamide, the rhodophyte

Table 2. Comparison of I_{50} values for acetazolamide and ethoxzolamide inhibition of carbonic anhydrase from *Prochloron*, human erythrocytes, spinach, pea, and various microalgal species.

Enzyme and source	I_{50} (μM)		
	Acetazolamide	*Ethoxzolamide*	*Reference*
Human Erythrocyte CA I	0.2	0.002	31
Human Erythrocyte CA II	0.01	0.002	31
Chlamydomonas Extracellular CA	0.002	0.005	9
Chlamydomonas Intracellular CA	300	20	21
Spinach CA	100	1	10
Pea CA	450	5	4
Prochloron CA	700	300	16
Synechococcus CA	–	50	6
Anabaena CA	0.3	0.003	53
Porphyridium CA	0.09	0.1	51

Porphyridium has CA localized mainly in the chloroplast [52]. CAs from higher plants, on the other hand, are generally considered to be relatively resistant to sulfonamides. Although there is evidence that cytoplasmic isozymes of CA are present in leaves of some plants, the majority of leaf CA activity in spinach is localized in the chloroplast [45, 50] specifically in the stroma [37]. The presence of transit peptide in cDNA coding for pea CA suggests that CA activity in pea also resides within the chloroplast [29]. With regards to the sulfonamide-resistant intracellular CA of *Chlamydomonas,* although a cytoplasmic form of enzyme exists, it was suggested that the observed I_{50} values probably correspond to the form of CA within the chloroplast [21]. Using immunological techniques, a 45 kilodalton polypeptide immunoreactive with the antispinach CA antiserum was detected in the chloroplast stromal fraction [21]. Recently, there was a report that CA associated with the chloroplast in *Chlamydomonas* is insoluble, suggesting that it is membrane-bound [40]. In another green alga, *Chlorella,* an insoluble membrane-bound CA which is associated with the chloroplast membranes was reported[38].

Since *Prochloron* exhibits appressed thylakoid membranes containing chlorophylls *a* and *b* characteristic of the chloroplasts of green algae and higher plants, some workers in the field of endosymbiosis favor the idea that the green algal chloroplasts may have arisen by the uptake of *Prochloron* as symbionts [49]. Comparison of the sequences of *psbA* genes, which encode the photosystem II thylakoid protein D1, from a related free-living, filamentous prochlorophyte, *Prochlorothrix,* with those reported for cyanobacteria, a green alga, a liverwort, and several higher plants places the prochlorophytes closer also to green plant chloroplasts than cyanobacteria [33]. On the other hand, sequence comparison of the genes encoding the 16S ribosomal RNA [39, 47], the large and small subunits of

RuBisCO [34] and a subunit of DNA-dependent RNA polymerase [36] places the prochlorophytes more closely related to cyanobacteria than to the green plastid lineage. Recently, on the basis of 16S ribosomal RNA data, it was suggested that prochlorophytes are polyphyletic within the cyanobacterial radiation, and not specifically related to chloroplasts [48].

The results presented in this paper showed that in terms of CA inhibition by sulfonamide, *Prochloron* is similar to both the unicellular cyanobacteria and to chloroplasts of green algae and higher plants. On this aspect, then *Prochloron* shares characters with both cyanobacteria and green chloroplasts, suggesting a possible link between the cyanobacteria and chlorophytes. Since sulfonamide inhibition of CA is attributed to its binding with the active site of the enzyme, it may be that the structures of active sites in CAs from *Prochloron, Synechococcus,* and chloroplasts of *Chlamydomonas,* spinach, and pea are quite similar to each other. When Western blot analysis was carried out to determine whether soluble protein extracts of *Prochloron* cross-react with anti-spinach CA antibody, a single immunosignal of approximately 34 kDa was observed (Fig. 2). However, concluding that this was *Prochloron* CA would be difficult since the antibody also reacted with some other proteins in the spinach soluble extracts.

At present, cDNAs coding for the spinach chloroplastic CA [11], pea chloroplastic CA [29] and *Chlamydomonas* extracellular CAs [18] have been isolated and characterized. No significant sequence similarity has been observed between these CAs [19]. More recently, a putative CA gene showing significant sequence similarity to spinach and pea chloroplastic CA but not to *Chlamydomonas* extracellular CA has been identified in *Synechococcus* [20]. Spinach CA and pea CA in the same paper were then suggested to be prokaryotic in nature whereas the *Chlamydomonas* extracellular CA, which shares sequence similarity with mammalian CAs (see also 18, 19) was suggested to be a eukaryotic type. Since *Prochloron* CA exhibits I_{50} values similar to spinach CA and *Synechococcus* CA but highly different from *Chlamydomonas* extracellular CA or mammalian CA, it would be of interest to determine whether *Prochloron* CA exhibits sequence similarity with the prokaryote-type CAs

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DNA SYSTEMATICS OF SIMAROUBACEAE *sensu lato:* PHYLOGENETIC AND TAXONOMIC IMPLICATIONS BASED ON THE CHLOROPLAST GENE *rbc*L

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ABSTRACT

Phylogenetic analyses of nucleotide sequence data for the chloroplast gene *rcb*L of representatives of all subfamilies indicate that the tropical plant family Simaroubaceae is, in any sense, polyphyletic. The family represents six separate evolutionary lineages, only three of which (Simarouboideae, *Harrisonia*, and Kirkioideae) belong in the Order Sapindales. The family is monophyletic only when composed of members of the subfamily Simaroubaceae plus Leitneriaceae, but excluding *Harrisonia*. Simaroubaceae in this strict sense belongs in a well-defined affinity group including Rutaceae, Cneoraceae, and Meliaceae, while *Harrisonia* has affinities with *Cneorum* and Rutaceae; all these members are characterized by the accumulation of triterpenoids. Kirkioideae occupies a basal position in the Sapindales within a weakly-defined clade including Anacardiaceae and Burseraceae. The other three lineages show affinities with taxa distant from Sapindales: *Irvingia* with a group of rosid I taxa (*sensu* Chase et al.) comprising in part members of Linales and Malphigiales; Surianoideae, including *Stylobasium*, forms a monophyletic group showing affinities with Polygalaceae and Leguminosae; *Picramnia* and *Alvaradoa*, cluster together in an isolated position between the broadly comprised groups of rosid I and rosid II. Support for the affinities suggested here is also evident in other data sources such as wood and pericarp anatomy, pollen morphology, and phytochemistry. The elevation of the picramnioid group, comprising *Picramnia* and *Alvaradoa*, to family rank is suggested, and the recognition of the segregate families, Kirkiaceae, Surianaceae (including *Recchia* and *Stylobasium*) and Irvingiaceae, is supported.

MARICULTURE OF THE SEA URCHIN *Tripneustes gratilla* AS A RESOURCE MANAGEMENT TOOL

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ABSTRACT

The collapse of the valuable sea urchin *Tripneustes gratilla* fishery in Bolinao, Pangasinan, has led to the formulation of alternative and multi-faceted approaches to sea urchin management which integrate scientific research and the active participation of local communities. In laboratory culture, the duration of sea urchin larval development ranged from 35-50 days after fertilization. Newly settled juveniles were reared in aquaria for 2-4 months until juveniles were about 1.5 cm test diameter (TD) in size. Thereafter they were transferred to sea pens maintained by local fishers. Juveniles grew rapidly at about 1.4 cm mo^{-1} and majority attained the minimum size of sexual maturity of 5-6 cm TC, only 7-8 months after artificial fertilization in the laboratory. Community-managed reproductive reserves in the form of sea pens or cages where juvenile sea urchins can be grown and selectively harvested (i.e., >7.0 cm TD) is an integral and viable part of the management strategy for the recovery of this valuable resource. The utility of this approach as a management tool is reviewed with respect to economic, ecological, and educational values based on the results of various field and laboratory studies and fishers' experiences. The adaptability of this approach, implications of the need for limited rights of exclusive use (e.g., sea pens/ cages) in a traditionally open access fishery, and the development of a local marketing system that would ensure equitable monetary returns to fishers in managing similar invertebrate resources are discussed.

INTRODUCTION

The fishery of the sea urchin *Tripneustes gratilla* is a major source of livelihood in many coastal villages in the Philippines. Its roe or gonads are eaten as a delicacy called "uni" in specialty restaurants in the country, and is a high-value export product to Japan, Korea, and Taiwan. Moreover, it is also a regular part of the diet of many local coastal communities (e.g., Northern Luzon). In recent years,

overexploitation has precipitated in the collapse of sea urchin fisheries in many areas nationwide. In Bolinao, Pangasinan, the commercial fishery for *T. gratilla* generated multimillion peso earnings per annum, and provided the major source of income for most coastal families prior to the collapse of the fishery in 1992. On January 1993, the local government issued an ordinance banning the commercial harvesting of sea urchins for one year to allow natural populations to recover. Three years after, despite the extended moratorium on commercial harvesting, natural recruitment of *T. gratilla* in the area became very weak. The once dominant invertebrate species on the reef flats of Bolinao is presently practically depleted.

The collapse of the *T. gratilla* fishery in Bolinao, led to the formulation of an alternative and multi-faceted approach to sea urchin management which integrates scientific research and the active participation of local communities. Community-managed sea pen culture which serves as mini-reproductive reserves and a supplemental source of livelihood is the centerpiece of this conceptual model for sea urchin management. Because of the poor natural recruitment of sea urchin populations in the area, the potentials of this management scheme can only be realized through the development of mass culture techniques for the artificial production of sea urchin seedstock. This presentation will describe the experimental and prototype mass culture set-up for larvae and the pilot grow-out culture of juveniles being developed at the UPMSI Bolinao Marine Laboratory. Some results of ongoing studies to enhance the growth and survivorship of cultured sea urchins at various stages of its development will be discussed.

LARVAL CULTURE AND DEVELOPMENT

In 1994, the entire life cycle of *T. gratilla* was completed under laboratory conditions at the Bolinao Marine Laboratory. Despite many previous attempts, this was the first time that the culture of sea urchin larvae and juveniles was successfully undertaken in the country. Moreover, F3 generations of laboratory cultured sea urchins are being used as broodstock in ongoing culture studies.

Cleavage is completed in 6-9 hours after artificial fertilization of the eggs. The prepluteus stage takes about two days, while the 2-arm, 4-arm, and 6-arm stages lasted about three, seven, and fifteen days respectively. The duration of the 8-arm stage is the most variable, ranging from fifteen to thirty days. On the average, the total larval duration ranged from 42-52 days. Moreover, high variability in the development rates was observed among individuals from the same batch of larvae as well as among larvae from different batches.

LARVAL SETTLEMENT AND JUVENILE GROWTH

Factors that may enhance settlement and metamorphosis of larvae are being investigated. Experiments on the effect of various primary films such as pure and disinfected cultures of the benthic diatom *Navicula ramossisima* and field-derived

films were conducted. Preliminary results indicate that substrates with field-derived films were the most effective inducers of settlement and metamorphosis. However, diatom-coated plates should also be provided to serve as a food source for the newly metamorphosed juveniles. Likewise, the effect of culture water conditioned with conspecific adults, a sympatric sea urchin species *(Salmacis sphaeroides)* and the brown alga *Sargassum* which is the major diet of juveniles and adults, is being investigated. Preliminary results indicate that the highest percentage of larvae that completely metamorphosed within the first six hours of the settlement assay occurred in *Tripneustes*-conditioned water. This result supports the hypothesis that the presence of conspecific adults is a settlement cue for larvae in the field. As such, the presence of conspecific adults should enhance the local recruitment of *T. gratilla* larvae.

Newly settled juveniles attained a size of about 1 cm test diameter (TD) after two to four months. Growth and survival rates of replicate groups of five batches of cultured sea urchins were monitored in laboratory tanks and in experimental sea cages at two sites in Bolinao (Lucero and Dewey). Results showed that growth and survival rates varied significantly with respect to rearing location and batch of sea urchins. In general, growth and survivorship were significantly higher for all batches of sea urchins reared in sea cages in Lucero and in laboratory tanks when compared to those reared in Dewey. Growth rates were highest during the first three months of the grow-out period prior to the attainment of sexual maturity. In Lucero and the laboratory, average monthly growth rates during this period ranged from 1.3-2.6 cm TD. Notably, majority of the juveniles in the grow-out experiments attained sexual maturity at a size of 5.0-6.0 cm TD, only 7-8 months after artificial spawning and fertilization in the laboratory. In contrast, average monthly growth rates ranged from 0.2-0.8 cm TD after attainment of sexual maturity.

PILOT COMMUNITY-BASED SEA URCHIN GROW-OUT CULTURE

Growing out juveniles to reproductive adults in sea enclosures is viewed as a complementary measure to enhance the recovery of natural stocks in two ways: aggregating conspecific adults increases the probability of successful fertilization in the field and enhances local recruitment of larvae. Laboratory-cultured seedstock (>1.0 cm TD, n = 1,200 juveniles) were provided to a local group of fishers who signified serious interest in undertaking grow-out culture of sea urchins primarily as a mini reproductive reserve. The fishers were responsible for providing the cages (i.e., at least the labor), feeding and maintaining the culture. Similar arrangements are being developed with other local fishers. This effort is being facilitated by an integrated community-based coastal resources management program which focuses on the empowerment of local communities to become effective resource managers and advocates of sustainable utilization of marine resources in Bolinao.

Aside from the apparent ecological and educational values, sea urchin grow-out culture is also a supplemental source of income for artisanal fishers. Local cooperators in Bolinao have agreed to selectively harvest cultured sea urchins which are > 7.0 cm TD to ensure that the urchins have already contributed to natural larval production. The viability of *T. gratilla* grow-out culture as a supplemental livelihood for fishers is very high because of the fast growth rates and early attainment of reproductive maturity of the species, the high local and export market value of sea urchin roe, and the low capital and maintenance cost of sea pens or cages.

Sea urchin grow-out culture can also be undertaken in areas where natural populations are not yet depleted, and natural seedstock are abundant. In this scenario the full potential of grow-out culture as a resource management tool should be emphasized as a means to educate resource users to become responsible partners in ensuring sustainable utilization of marine resources in general. A similar management approach may be used for other exploited benthic invertebrate resources in the country to forestall further depletion of natural stocks.

THE COMPLEX OF PHILIPPINE *GYNAIKOTHRIPS* SPECIES

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ABSTRACT

Gynaikothrips are medium-sized to large thrips distributed mainly in the Orient with a few species known from the new world. There are six known species of *Gynaikothrips* in the Philippines: *Gynaikothrips uzeli* Zimmermann, *Gynaikothrips luzonensis* Priesner, *Gynaikothrips capitulatus* Reyes, *Gynaikothrips pedanus* Reyes, *Gynaikothrips pontis* Reyes, and *Gynaikothrips xynos* Reyes.

The species of *Gynaikothrips* in the Philippines can be placed into four species groups: (a) *uzeli* group: *including G. uzeli*, (b) *luzonensis group:* including *G. luzonensis*, (c) *pedanus* group: incuding *G. capitulatus* and *G. pedanus*, and (d) *pontis* group: including *G. pontis* and *G. xynos*. The first three species groups possess adult characters which definitely place them under *Gynaikothrips* while the *pontis* group possess characters that grade from those of adult *Gynaikothrips and Gigantothrips.*

INTRODUCTION

The name *Gynaikothrips* was first introduced by Zimmermann in 1900 as a misstatement for *Mesothrips.* In Zimmermann's Figure 4 (Zimmermann 1990), the caption appeared as *Gynaikothrips uzeli.* In 1911, Karny gave the first diagnosis of genus *Gynaikothrips* (Stannard 1957; Jacot-Guillarmod and Brothers 1986).

Gynaikothrips are medium-sized to large thrips distributed mainly in the Orient with a few species known from the new world. Members of most species are associated with various species of *Ficus* where some of them produce leaf galls (Reyes 1994).

Gynaikothrips Zimmermann
Type species: *Mesothrips uzeli* Zimmermann, 1900: 12, by monotypy

Diagnosis:
Antennae. Antennae 8-segmented; intermediate antennal segments slender, moderately long; segment III with 1 sense cone; segment IV with 2 or 3 sense cones.

Head. Head elongate, to rectangular, reticulate. Eyes moderately large, close together. Postocular setae minute to developed. Maxillary stylets short to retracted halfway into head capsule or extended level of postocular setae. Mouthcone elongate, broadly rounded.

Thorax. Pronotum with sculpture of irregular twisted striae or weak hexagonal reticulations; epimeral sutures complete or incomplete. Praepectal plates absent; mesopraesternum well developed.

Legs. Forefemora slender; foretarsi without tooth in both sexes.
Wings. Forewings parallel-sided, with duplicated cilia.

Abdomen. Pelta triangular to hat-shaped, small to moderate in size, occasionally with 2 additional lateral lobes. Apical abdominal tergites elongate; tergites II-VII each usually with 2 pairs of wing retaining setae. Tube long, often hairy and slightly bowed.

Key to Philippine Species of *Gynaikothrips* Zimmermann

1	Antennal segments robust; head slightly longer than wide; eyes prolonged ventrally	*G. pedanus Reyes*
1	Antennal segments moderately elongate, head longer than wide; eyes not prolonged ventrally	2
2(1′)	Abdominal tergites III and IV each with 3 or more pairs of wing retaining setae	3
2	Abdominal tergites III and IV each with 2 pairs of wing retaining setae	4
3(2)	Maxillary stylets reaching level of postocular setae, lying close together towards the base of head; B1 setae of abdominal tergite IX short and stout, rounded apically	*G. pontis Reyes*
3	Maxillary stylets short, lying almost parallel to each other at the base of the head; B1 setae of abdominal tergite IX long and slender, almost pointed apically	*G. xynos Reyes*

4(2') Posteroangular setae of pronotum reduced; head about 1.2 times as long as wide; maxillary stylets barely retracted into base of head ..... *G. capitulatus Reyes*

4' Posteroangular setae of pronotum well developed; head at least 1.5 times as long as wide; maxillary stylets at least about one third as long as head 5

5(4') Postocular setae short, about 0.6 times as long as eyes; head widest towards base ..... *G. uzeli* Zimmermann

5' Postocular setae about as long as eyes or longer; head slightly constricted towards base ..... *G. luzonensis* Priesner

UZELI GROUP

1. *Gynaikothrips uzeli* Zimmermann
Mesothrips uzeli Zimmermann, 1900: 12

Diagnosis:

Antennae. Antennal segments III slightly longer and more slender than IV: segment III yellow; IV to VI yellow with brown apices; VII brown with pale base; VIII brown.

Head: Head rectangular, surface reticulate and with or without small warts. Postocular setae reduced, shorter than length of eyes.

Thorax: Pronotal epimeral setae longest among major setae; anteromarginal setae minute.

Legs. Legs generally brown, with pale apices.

Wings. Forewings pale with duplicated cilia.

Abdomen. Pelta triangular. Abdominal tergites II to VII each with two pairs of wing retaining setae. Tube longer than head.

This species is known in Indoensia, Malaysia, Singapore, Vietnam, India, and Laguna, Philippines.

G. uzeli, the type species of genus *Gynaikothrips*, are difficult to differentiate from those of *G. ficorum*, which are also common on several species of *Ficus*.

LUZONENSIS GROUP

1. *Gynaikothrips luzonensis* Priesner
Gynaikothrips luzonensis Priesner, 1939: 480

Diagnosis:

Antennae. Antennal segment II longer and more slender than segment IV.

Head. Head longer than wide, with four pairs of small median setae. Ocellar hump prominent, eyes large. Postocular setae longer than dorsal length of eyes, with blunt apices.

Thorax. Pronotal major setae reduced with knobbed apices; anteromarginal setae minute.

Legs. Legs bicolored.

Wings. Forewings pale, each with 18-19 duplicated cilia.

Abdomen. Pelta triangular. Abdominal tergites II and VII each with two pairs of wing retaining setae. Tube longer than head.

Adults of this species differ from those of *G. uzeli* in the shape of the head and pelta, well-developed postocular setae, and presence of four pairs of small median setae in the head.

G. luzonensis is known in Taiwan and in Laguna and Zamboanga, Philippines. They were collected on different species of *Ficus, Antidesma,* and *Ricinus communis.*

PEDANUS GROUP

1. *Gynaikothrips capitulatus* Reyes
   Gynaikothrips capitulatus Reyes, 1996: 89.

2. *Gynaikothrips pedanus* Reyes
   Gynaikothrips penadus Reyes, 1994: 397.

Members of this species group are characterized by the following features:

Diagnosis:

Antennae. Antennal segments robust to elongate; segment III with 1 inner sense cone; segment IV with 1 inner and 2 outer sense cones

Head. Head longer than wide, reticulate. Eyes about a third of head length sometimes prolonged ventrally. Postocular setae developed, shorter than dorsal length of eyes. Maxillary stylets short.

Thorax. Pronotal major setae reduced to well developed. Anteromarginal setae vestigial to developed. Epimeral setae prominent.

Legs. Legs bicolored; femora brown sometimes with pale apices; foretibiae yellow; mid and hindtibiae brown with pale apices; tarsi yellow; foretarsi without tooth.

Wings. Forewings light brown, with 8 to 14 duplicated cilia; subbasal wing setae well-developed, with blunt to knobbed apices; setae S3 longest.

Abdomen. Pelta triangular, reticulate; a pair of companiform sensilla present. Abdominal tergites II to VII each with 2 pairs of wing retaining setae. B1 setae of tergite IX shorter than tube, pointed at apex. Tube longer than head.

G. capitulatus differ mainly from *G. pedanus* by the shape of their antennal segments and pelta, and in having vestigial anteromarginal setae. This species is known only in Zamboanga, Philippines on an unidentified plant.

Unlike all other described members of the genus, adults of *G. pedanus* species have a short head, stouter body, and more robust antennal segments. As in *G. capitulatus* they possess short maxillary stylets. This species is known in Cagayan, Philippines on rolled leaves of Curran's Lipote.

PONTIS GROUP

1. *Gynaikothrips pontis* Reyes
   Gynaikothrips pontis Reyes, 1996: 92

2. *Gynaikothrips xynos* Reyes
   Gynaikothrips xynos Reyes, 1996: 94

Members of this species group are characterized by the following features:

Diagnosis:

Antennae. Antennal segments III-VIII elongate; segments I and II brown, II pale basally or apically; III to VIII yellow or brown; III and IV subequal, III with 1 outer sense cone, IV with 1 inner and 1 or 2 outer sense cones.

Head. Head rectangular, about 2.0 times as long as wide, widest at base, reticulate with small warts. Eyes large, protruded. Postocular setae reduced to well-developed, with blunt apices. Maxillary stylets short to reaching level of postoculars.

Thorax. Pronotal major setae with blunt apices; posteroangular setae reduced to developed; epimeral setae well developed.

Legs. Femora shown; tibiae yellow or brown on basal third; tarsi yellow; foretarsi with tooth.

Wings. Forewings pale, with dark, median longitudinal stripe and duplicated cilia.
Abdomen. Pelta hat-shaped to triangular. Abdominal tergites with strong warty reticulations. Tergite II with 2 pairs of wing retaining setae, those on III to V with 4 pairs, VI to VII 2 pairs. Tube longer than head.

G. xynos differs mainly from *G. pontis* in having shorter maxillary stylets lying nearly parallel in the base of head, conspicuous postocellar setae, shorter antennal segment IV with 1 inner and 2 outer sense cones (1 outer sense cone in *G. pontis),* triangular pelta; and long and slender B1 setae on tergite IX. G. pontis is known only in Laguna and Mt. Apo, Philippines on leaves of *Ficus pseudopalma,* an unidentified plant, and on sweeping materials. *G. xynos,* on the other hand, is known only in Laguna, Philippines on *Euphorbia hirta* and *Ficus* sp.

G. pontis and *G. xynos* resemble *Gigantothrips elegans* Zimmermann, type species of the genus *Gigantothrips* in having longer body and intermediate antennal segments; tergites with additional pairs of wing retaining setae and accessory setae; and presence of foretarsal tooth.

G. pontis and *G. xynos* differ from *G. elegans* in having antennal segment III shorter or about as long as segment IV; pronotum with less-developed setae anteriorly and laterally except anteroangulars; abdominal tergites II with 2 pairs of wing retaining setae, III to V with 4 pairs, and VI and VII with 2 pairs. In *G. elegans*, abdominal tergite II with 4 pairs of wing retaining setae, III to VII with 5 or more pairs of such setae.

Gigantothrips elegans Zimmermann
Type species: *Gigantothrips elegans* Zimmerman, 1900: 18 by monotypy.

DIAGNOSIS: Head elongate, to rectangular, reticulate. Eyes moderately large, closed together. Postocular setae minute to developed. Antennae 8-segmented; intermediate antennal segments slender, moderately long; segment III with 1 sense cone; segment IV with 2 or 3 sense cones. Maxillary stylets retracted into head capsule. Mouthcone elongate, broadly rounded.

Pronotum with sculpture of irregular, twisted striae or weak hexagonal reticulations; epimeral sutures complete or incomplete. Praepectal plates absent; mesopraesternum well developed. Forefemora slender; foretarsi each with small tooth in both sexes. Forewings parallel-sided, with duplicated cilia.

Pelta hat-shaped, moderate in size. Abdominal tergites slender, elongate; tergite II with 4 pairs of wing retaining setae, III-VII each with 5 or more pairs of wing retaining setae. Tube long, with developed setae.

Worldwide, about 21 species are presently included in genus *Gigantothrips* and about 89 species in genus *Gynaikothrips*. Most of these species are known in the tropics and usually associated with various species of *Ficus*.

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