Detection of feeding deterrents and phycotoxins from marine phytoplankton using a new bioassay technique - ecological and commercial applications

B.A. Shaw[1,2], R.J. Andersen[1] and P.J. Harrison[1]

1 University of British Columbia, Department of Oceanography, Vancouver, B.C., V6T 1Z4, Canada
2 Present address: Northwest College, 130 First Ave. West, Prince Rupert, B.C., V8J 1A8, Canada

It has been known for the last three decades that some marine phytoplankton can produce chemical compounds, referred to as feeding deterrents, which make them unpalatable to zooplankton, and thus protect them from grazing. There have been two main approaches to studies on feeding deterrents: (1) a number of species of phytoplankton have been demonstrated to reduce feeding of certain zooplankton; and (2) several well-known phycotoxins have been shown to be feeding deterrents. However, very little research has been done on the chemistry and physiology of feeding deterrent compounds, and no feeding deterrent compound has been isolated and structurally characterized from a marine phytoplankton using a bioassay specifically designed to detect feeding deterrents.

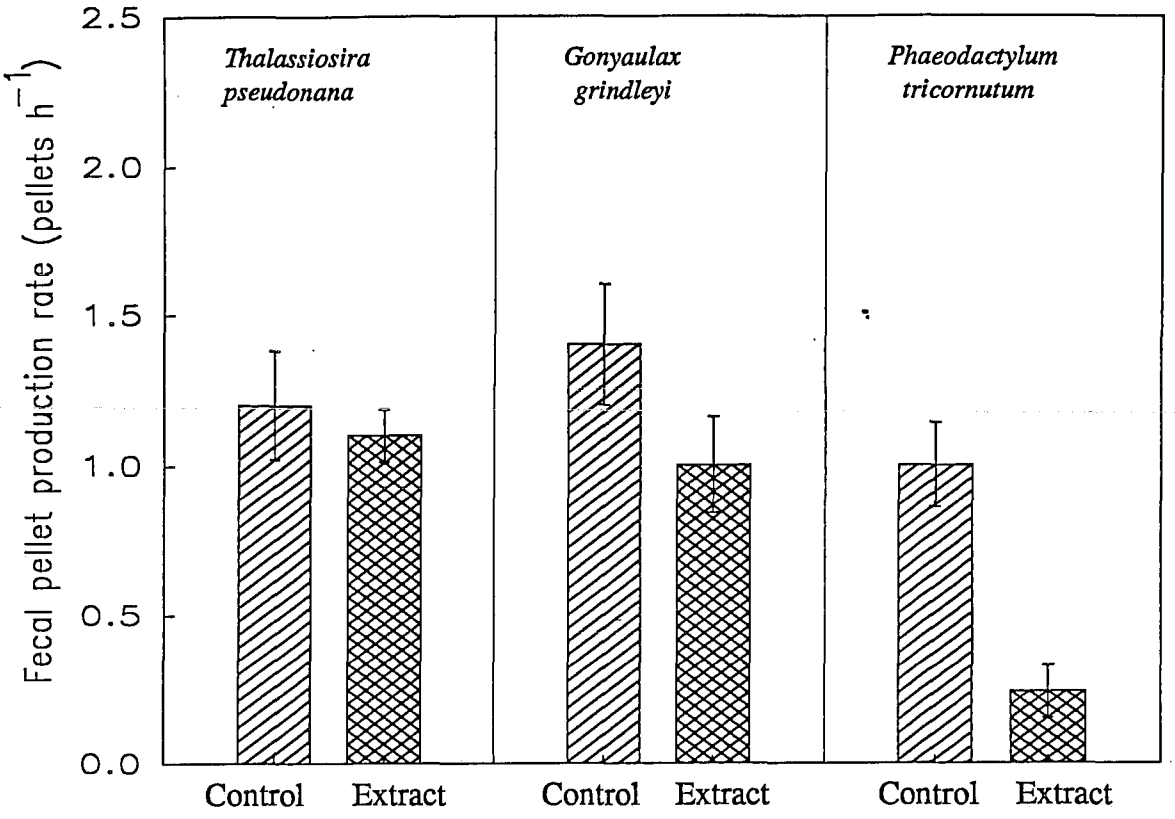
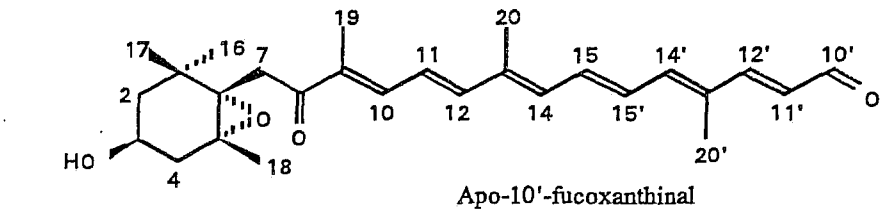


Figure 1

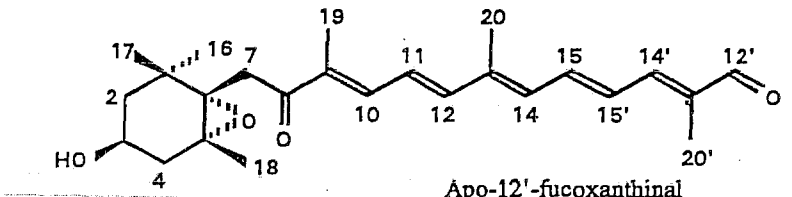
Feeding deterrent bioassay results for cellular extracts of three marine phytoplankton, *Thalassiosira pseudonana*, *Gonyaulax grindleyi*, and *Phaeodactylum tricornutum*.

In order to screen phytoplankton species for feeding deterrent production and to isolate and identify feeding deterrent compounds, a new, rapid, and reliable laboratory bioassay was developed. This bioassay used the harpacticoid copepod *Tigriopus californicus*, and measured inhibition of feeding by measuring the fecal pellet production rate. Using this bioassay, cellular extracts from the diatom *Phaeodactylum tricornutum* and the dinoflagellate *Gonyaulax grindleyi* gave feeding deterrent responses, while extracts from the diatom *Thalassiosira pseudonana* gave no feeding deterrent responses (Fig. 1). Live *P. tricornutum* cells also deterred feeding at densities of 6×10^5 cells mL^{-1}. Feeding deterrent compounds were isolated and characterized from *P. tricornutum* using bioassay-guided chemical fractionation. Spectroscopic techniques identified the isolated compounds as apo-10'-fucoxanthinal, apo-12'-fucoxanthinal, apo-12-fucoxanthinal, and apo-13'-fucoxanthinone (Fig. 2).

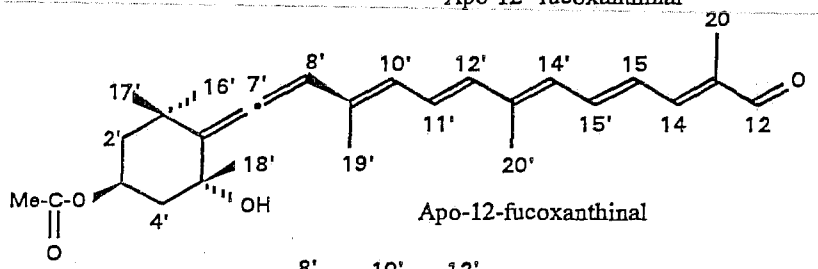
Preliminary studies on the physiology of production of these feeding deterrents were performed. In order to carry out these studies, an analytical HPLC method was developed to measure the apo-fucoxanthinoid concentrations in crude cell extracts. The apo-fucoxanthinoids were shown to be produced by *P. tricornutum* when the cells entered senescence due to phosphate limitation. As senescent cells are more susceptible to predation, the production of feeding deterrents during senescence may protect these cells from grazing until the limiting nutrient is replenished.



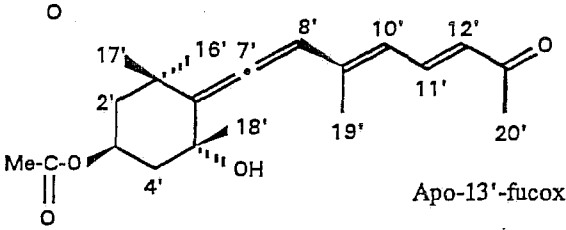
Apo-10'-fucoxanthinal



Apo-12'-fucoxanthinal



Apo-12-fucoxanthinal



Apo-13'-fucoxanthinone

Figure 2

Apo-fucoxanthinoid feeding deterrent compounds isolated from the marine diatom *Phaeodactylum tricornutum*

The toxic and feeding deterrent effects of the apo-fucoxanthinoids and several phycotoxins (okadaic acid, domoic acid, and microcystin-LR) on the copepod *Tigriopus californicus* were studied. IC50 (feeding inhibition) and LC50 (toxicity) curves were generated from these experiments using the equation:

$$y = y_0 e^{-kx^a}$$

For those compounds showing both feeding deterrent and toxic effects, the IC50 and LC50 curves were deconvoluted if they overlapped. Each compound was then classified as either toxin, feeding deterrent, both toxin and feeding deterrent, or inactive. The concentration of apo-fucoxanthinoids necessary to inhibit feeding of *T. californicus* by 50% ranged from 8.6 to 60 μM and was ≈ 1000 times lower than the concentration of total intracellular apo-fucoxanthinoids in *Phaeodactylum tricornutum*. These compounds are probably effective feeding deterrents at low concentrations such as would be expected in the natural environment. Three of the compounds tested (apo-12-fucoxanthinal, apo-13-fucoxanthinone, and microcystin-LR) showed only feeding deterrent effects in the range 0 to 50 μM, while domoic acid showed only toxic effects in this range, and the other compounds tested showed both feeding deterrent and toxic effects (Fig. 3). Compounds with only

feeding deterrent effects are probably detected by the copepod's chemoreceptors and not ingested, while compounds showing toxic effects are probably ingested, resulting in mortality. Thus, the bioassay developed for this research not only provides a valuable tool in screening phytoplankton for feeding deterrent compounds, and studying the chemistry and physiology of these compounds, but also to differentiate between toxic and non-toxic responses.

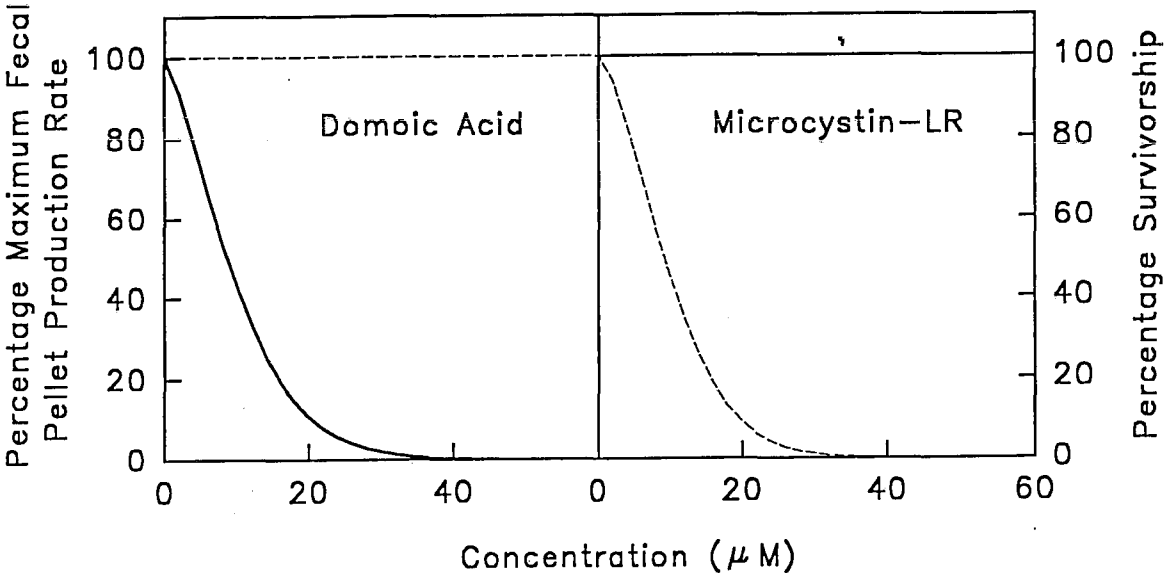


Figure 3

Comparison of LC50 and IC50 curves calculated from data for the effects of domoic acid and microcystin-LR on the copepod *Tigriopus californicus*. Solid line is the LC50 curve; dashed line is the IC50 curve.

Research on feeding deterrents and phycotoxins has major ecological significance. Screening various phytoplankton for feeding deterrent activity will determine which species of phytoplankton produce feeding deterrents. If phycotoxins have feeding deterrent activity, this may explain the purpose of the production of phycotoxins by phytoplankton in the natural environment. In addition to being potential human health problems, production of feeding deterrents by certain species of phytoplankton may control grazing and determine which species of phytoplankton will bloom and how long the bloom will persist. Thus, feeding deterrents may play an important role in phytoplankton species succession. Ultimately, these compounds may control the transfer of energy along some paths in the marine food web.

The results of this research may also have commercial implications. Phytoplankton are an important food source in mariculture. They are an essential component in the diet of marine bivalve molluscs (e.g. oysters, clams, scallops, and mussels), the larvae of some marine gastropods (e.g. abalone), larvae of salt-water shrimp (*Penaeus* and *Metapenaeus*), and zooplankters. Zooplankters, in turn, can be used as live food for rearing larvae of crustaceans (prawns, shrimp, crabs, and lobsters). Commonly used zooplankters are rotifers (*Brachionus*) and copepods

(*Tigriopus*). Today, more than 40 different species of phytoplankton are being used in mariculture. The food quality of these species varies greatly and experienced aquaculturalists frequently refer to 'good' and 'poor' food quality species. Production of feeding deterrents by 'poor' species may be the reason for decreased growth rates or increased mortality in mariculture species (e.g. oysters). Additionally, many mariculture facilities use natural, filtered seawater drawn from a local source. If this source is contaminated by blooms of phytoplankton which produce extracellular feeding deterrents, then the seawater itself may lead to decreased growth in the cultured organisms. Oyster beds and fish net pens exposed to blooms of feeding deterrent producing phytoplankton may also suffer decreased feeding resulting in decreased growth and health. A better understanding of which species produce feeding deterrents and what factors affect the production of these feeding deterrents would improve culturing practices in mariculture. Specifically, this research has shown that the diatom *Phaeodactylum tricornutum*, which is commonly used in mariculture, produces feeding deterrents and is not recommended for used as a food species, and that several common local species (*Gonyaulax grindleyi*, *Pseudonitzschia pungens*) produce compounds which may be harmful to exposed mariculture organisms.