X FE

Discriminating Populations of the Eastern King Prawn, *Penaeus plebejus*, From Different Estuaries Using ICP-MS Trace Element Analysis

A. J. Courtney, D. J. Die, and M. J. Holmes
Queensland Department of Primary Industries
Southern Fisheries Centre
P.O. Box 76 Deception Bay
Queensland 4508, Australia

ABSTRACT

ICP-MS is being used to determine if prawns, like some species of fish, possess an "environmental imprint" attained in their juvenile nursery habitats. Such an imprint could act as a naturally occurring tag and provide useful information on nursery grounds, migration patterns, and stock structure. Samples of juvenile eastern king prawns, *Penaeus plebejus*, from four different estuarine nursery areas several hundreds of kilometers apart were distinguished from one another using combinations of the concentration of elements in their body tissues. Four different body tissues were used: eyes, hepatopancreas, abdominal muscle, and exoskeleton.

Canonical-variate (discriminant) analyses showed that each of the four body tissues could be used to correctly classify the samples with high (100%) predictability. The results, although helpful, should be treated with caution as they only provide a static and narrow representation of the prawns' elemental profiles in space and time. Further experiments are in progress to determine if the prawns retain these differences through time in the wild and in the laboratory.

INTRODUCTION

Fisheries biologists and managers rely heavily on the results of tagging studies to estimate growth and mortality, and to provide information on movement and stock structure. The accuracy of these parameters is imperative to the success of any production model used to optimize the resource. Identification, usually through some form of tagging, is also required to assess the success of restocking programs. Tagging, however, can have inadvertent and detrimental effects on the individuals and can bias estimates of the parameters being measured. Penn (1975) and Hill and Wassenberg (1985) have noted some of the effects that tagging has on molting, growth, and activity of marine prawns. Ideally, for any tagging study, what is sought is a completely benign tag that is unique to the individual (or group of individuals), readily identifiable, and retained through time.

Edmonds et al. (1989,1991) concluded that the concentration of trace elements deposited in the sagittal otoliths of certain marine teleosts reflect an environmental history of individual fish. Fish from various geographic regions could be grouped according to the concentration of certain trace elements. These differences were used to support concepts of nonmixing groups and stock structure. Although crustaceans do not possess permanent bony structures, Whyte and Boutillier (1991) found that the concentrations of elements in the carapace of female spot prawns, *Pandalus platyceros*, differed substantially in individuals from separate geographic locations.

Eastern king prawns, *Penaeus plebejus*, are one of the most valuable fisheries species on the east coast of Australia. Tagging studies (Ruello 1975, Potter 1975, Glaister et al. 1987, Montgomery 1990) have revealed that *P. plebejus* undertakes extensive migrations, sometimes exceeding 1000 km, in a northerly direction and from shallow to deep water against the East Australian Current. This migratory behavior results in the prawns crossing state jurisdictional boundaries and complicating management of the fishery. The importance of individual rivers, estuaries, and embayments as nursery grounds and sources of recruitment to offshore stocks is largely unknown. If the prawns were to acquire and retain some type of "environmental imprint," similar to that laid down in the fish otoliths (Edmonds et al. 1989, 1991), then such an imprint could prove to be of value in determining an individual's geographic nursery origin and important areas for recruitment in the fishery.

To be of value to fisheries managers, an environmental imprint must be retained in the prawn through time and be independent of any subsequent change in habitat. Secondly, the imprint must be detectable in adults, or older stages that may have migrated hundreds of kilometers over several months. The body tissues in Crustacea that are most likely to retain an environmental imprint are unknown. It is the objective of this paper to assess inductively coupled plasma-mass spectrometry (ICP-MS) as a means of detecting elemental profiles or combinations of elements, in several prawn tissues that have the potential to be used as environmental imprints.

MATERIALS AND METHODS

Field Sampling

Samples of juvenile *P. plebejus* were obtained at night from four estuarine nursery areas (lower reach of the Elliott River and Deception Bay, Queensland, and the lower reaches of the Tweed and Clarence Rivers in northern New South Wales) on the East Coast of Australia from September 22–October 15, 1992 (Figure 1). About 120 prawns of similar size and ages were sought from each area using a 1-m beam trawl with a 2-mm mesh nylon net attached. The beam trawl was towed along the bottom in approximately 3 m of water using a 5-m aluminum dinghy. In order to minimize stress and mortality on the prawns, the duration of the trawl tows did not exceed five minutes.

A number of steps were adopted to minimize contaminating the prawns and thereby, altering their elemental concentrations. These included sorting the catch on board on a clean plastic surface that had been scrubbed and washed in the surrounding seawater, and handling the prawns with disposable rubber gloves that were also washed in the surrounding seawater. Juvenile *P. plebejus* were quickly identified from other prawn species and placed in plastic 60-liter drums that had been scrubbed, washed, and flushed extensively in seawater. Portable aerators were used to pump air into the seawater in the drums. Air stones were not used to filter and disperse the air as this was considered to be a possible source of contamination. Instead, transparent plastic hosing with numerous tiny puncture holes and sealed at one end were used to aerate the surface of the water.

Laboratory Preparation

The prawns were held in the aerated drums for a minimum of 12 hours after capture and transported live to the laboratory within 48 hours. This allowed the prawns to purge their guts of ingested matter, and it ensured that they were anatomically intact upon arrival. None of the internal body organs, such as the hepatopancreas which ruptures easily, had been damaged or broken down which could have resulted in leaching elements and contaminating other body tissues. Upon arrival at the laboratory, the prawns were individually weighed, placed into plastic storage containers that had been washed in 10% nitric acid, and then killed by chilling in a freezer.

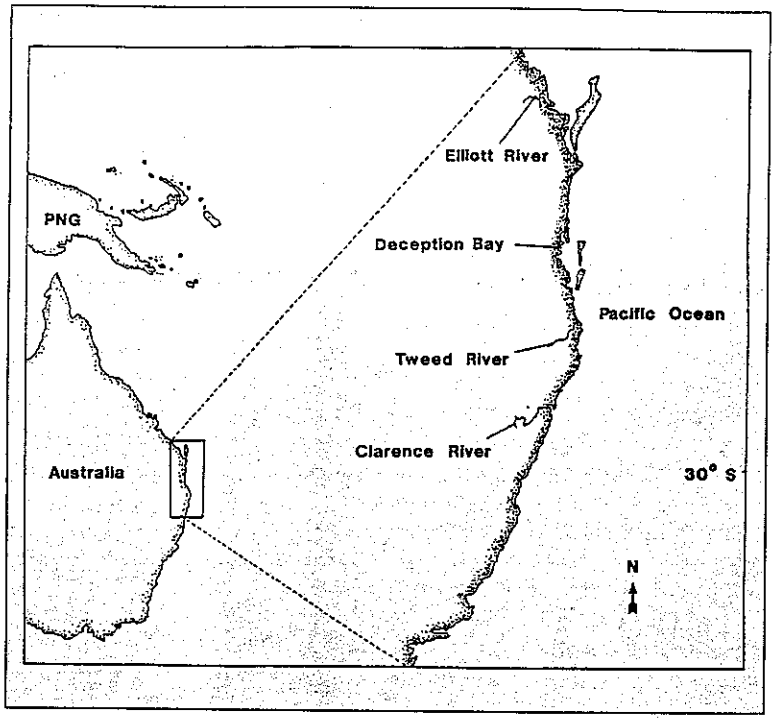

Fig. 1. *The East Coast of Australia showing the positions of the four juvenile eastern king prawn nursery areas sampled during the study.*

Dissections were carried out in a laminar flow cabinet while the prawns were still frozen, using stainless steel scissors, forceps, and a scalpel. A team of two undertook the dissections with each person using a separate set of dissecting instruments. Four body tissues were dissected out to assess their potential to yield a suitable imprint: eyes, hepatopancreas, abdomen with the hindgut removed, and abdominal exoskeleton (shell). For each prawn, the cephalothorax (head) was cut off and given to one person whose task was to remove the eyes (at the base of the compound eye, not including the eye stalk) and cut out the hepatopancreas. The other person received the abdominal half of the prawn and removed carapace and hindgut from the abdominal muscle. Ten samples of each tissue were obtained from each nursery area (10 samples each for abdomen muscle, hepatopancreas, eyes, and abdominal exoskeleton). Each sample contained pooled tissue from 10 or 12 prawns, depending on the number caught in the particular area. Samples were then placed in labeled plastic vials.

To determine if the elemental concentration was independent of prawn size, surplus individual prawns were left intact and analyzed as "whole prawns."

Preparation for ICP-MS

All analyses were carried out on a standard Perkin-Elmer® Sciex ELAN™ 5000 Inductively Coupled Plasma-Mass Spectrometer (ICP-MS). All sample vials and plasticware used for the preparation of standard solutions, dilution, and storage of sample digests were soaked in nitric acid (10%) for a minimum of 48 hours. All items were then rinsed three times in reverse osmosis prepared water, followed by three further rinsings with polished reverse osmosis (ROP) prepared water (18 MΩ). All borosilicate glass volumetric flasks were fitted with PTFE stoppers. These flasks and the PTFE beakers were refluxed with concentrated nitric acid for eight hours, allowed to cool, rinsed three times in RO water, and then soaked and cleaned as per the plasticware. Nitric acid was purified by sub-boiling double distillation of reagent-grade feedstocks in quartz stills.

Mixed multielemental standard solutions were prepared from 1000 ng/L stock solutions. Aluminum standards were prepared separately in TPX (polymethylpentane) volumetric flasks. The adsorption/release equilibriums of glass with aluminum in solution make low level determination of this element in glass highly inaccurate. Sample dissolution was achieved using a nitric acid microwave-assisted digestion. The system used was a Microwave Laboratory Systems MLS 1200, manufactured by MILESTONE, Italy. The frozen samples were placed in a DynaVac Freeze Drying Unit (Model: FD2) and allowed to dry to constant weight. Samples were then ground to a fine powder to achieve a homogeneous final product. Of this material, 100–200 mg was accurately weighed into a TFM™ [Tetrafluormethoxil, a PTFE (Teflon)-based material] insert of the microwave digestion system. Nitric acid (4 mL) was added and the vessels sealed and placed in the microwave oven. The oven program used was as follows:

(a) 250 watts for eight minutes

(b) 400 watts for four minutes

(c) 250 watts for four minutes

It should be noted that 250 watts power with this system is a continuous energy output which results in more even and controlled heating, producing a gradual pressure increase to a maximum of 30 bar. The vessels were then removed from the oven and cooled in an ice-bath for a minimum of one hour. This step is necessary to avoid losses of the sample from aerosols released upon opening of the vessels.

The sample solution was then transferred to a PTFE beaker and made up to 15.0 g with ROP water. Of this solution, 1.5 g was transferred to a polypropylene tube and set aside for mercury determination. A further 6.0 g of the sample solution was placed in a second polypropylene tube and stored as a replacement if required. The remaining 7.5 g of the sample solution in the beaker was taken to near dryness on a ceramic hot plate at 90°C. An additional 2 mL of nitric acid and 0.2 mL of hydrogen peroxide (30% w/v) were added dropwise and again taken to near dryness. This step was included to ensure complete digestion and to remove volatile interfering matrix components. The digestion solution was washed into a 50-mL polypropylene tube using 1% nitric acid, accurately made up to 20.0 g and used for the solution nebulization ICP-MS. The procedure is represented diagrammatically in Figure 2.

Instrumental ICP-MS Conditions

The plasma flow, nebulizer flow, and auxiliary flow were set at 15 L/min, 0.92 L/min, and 0.8 L/min, respectively. The RF power was 1000 watts, and the CEM voltage was 0 kV. The sample uptake was set at 1 mL/min.

Statistical Methods

Prawns from the different nursery habitats were treated as separate groups. In order to distinguish between groups, canonical-variate (discriminant) analyses were undertaken, treating the concentration of each element as a discriminating variable. The statistical graphics software package STATGRAPHICS was used to transform and analyze the data.

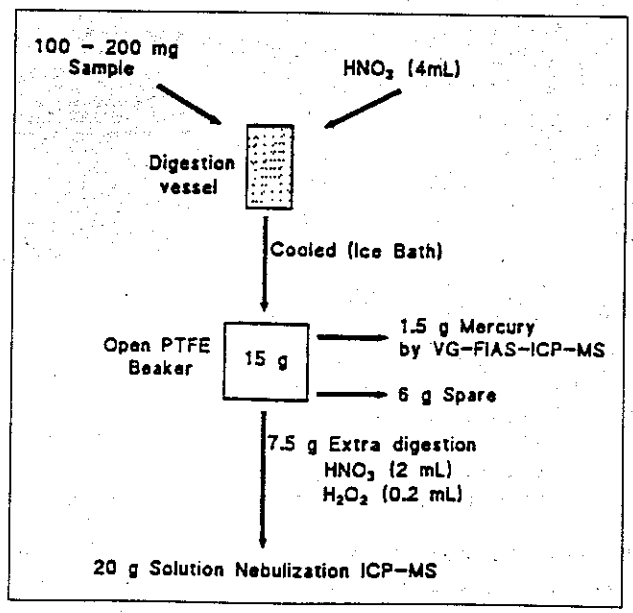

Fig. 2. *Diagrammatic representation of tissue for ICP-MS analyses.*

Sciex is a registered trademark and ELAN is a trademark of SCIEX, a division of MDS Health Group.
Perkin-Elmer is a registered trademark of The Perkin-Elmer Corporation.

RESULTS AND DISCUSSION

We attempted to sample prawns of similar size from each of the four areas to reduce attributing any detectable differences between the areas to differences in prawn size. However, it was not possible to control the exact size or abundance of prawns trawled, and consequently, there was a significant difference (ANOVA $p < 0.01$) in the mean weights of the prawns from each area (Table I).

We then determined whether these differences could influence the overall results by examining possible relationships between elemental concentration and prawn size, using "whole prawns" from several of the sampling areas, ranging in size from approximately 0.3 g to 3.0 g, and several elements such as sodium, aluminum, iron, cadmium, strontium, copper, chromium, caesium, molybdenum, selenium, rhodium, and arsenic. None of the regression analyses were significant ($p > 0.05$). This suggests that for the size range prawns sampled, the concentration of elements was independent of size. Thus, any detectable differences between samples from different nursery areas was unlikely to be due to differences in the size of the prawns.

The canonical variate analyses showed that the prawns could be separated using the concentrations of 15 elements (Table II) in their tissues and that each of the four body tissues was suitable for discriminating (Figure 3a–d). The analyses correctly predicted, for each tissue type, from which nursery area each of the 40 samples was obtained (100% discrimination).

Although the concentration of elements and compounds in various body tissues is known to vary with molt stage (Greenway 1985) and reproductive condition (Galois 1984, Anderson et al. 1985) in Crustacea, it is unlikely that the present results were influenced by such differences in prawn condition. First, the prawns examined were very young, probably no more than four weeks old, and thus all at the same stage of maturation (immature). Second, there was no evidence of synchronized molting, within or between sample areas. After each night of sampling, there were several exuviae (molts) floating on the surface of the water in the 60-L holding drums, indicating that a percentage of any particular population molt every day. Thus, it would be misleading to attribute the observed differences to differences in molt stage between areas.

The results are encouraging because they imply that prawns sampled from different estuarine nursery areas can be distinguished from one another using their elemental compositions. However, the results should also be interpreted with caution, because it is unknown whether these differences are retained in the prawns through time, as they grow, migrate, and undergo reproductive cycles. Furthermore, it is also unknown

TABLE I
Number and Mean Size of Juvenile Eastern King Prawns, *Penaeus plebejus*, Sampled From Four Nursery Habitats From the East Coast of Australia

Estuary/ nursery area	Number of prawns	Mean weight (g)	Standard deviation
Elliott River (Queensland)	120	0.6935	0.2261
Deception Bay (Queensland)	100	1.1136	0.3294
Tweed River (New South Wales)	120	0.7275	0.2482
Clarence River (New South Wales)	120	0.7743	0.2965

TABLE II
Elements Used for Each Tissue Type as Discriminating Variables to Identify Juvenile Eastern King Prawns, *Penaeus plebejus*, from Different Nursery Habitats

Eyes	Hepatopancreas	Abdomen muscle	Exoskeleton
Sodium (Na)	Sodium (Na)	Sodium (Na)	Sodium (Na)
Magnesium (Mg)	Magnesium (Mg)	Magnesium (Mg)	Magnesium (Mg)
Phosphorus (P)	Caesium (Cs)	Phosphorus (P)	Phosphorus (P)
Sulphur (S)	Sulphur (S)	Sulphur (S)	Sulphur (S)
Potassium (K)	Potassium (K)	Potassium (K)	Potassium (K)
Calcium (Ca)	Calcium (Ca)	Calcium (Ca)	Calcium (Ca)
Copper (Cu)	Copper (Cu)	Copper (Cu)	Vanadium (V)
Barium (Ba)	Zinc (Zn)	Barium (Ba)	Cobalt (Co)
Strontium (Sr)	Strontium (Sr)	Strontium (Sr)	Strontium (Sr)
Manganese (Mn)	Boron (B)	Caesium (Cs)	Ytterbium (Y)
Selenium (Se)	Selenium (Se)	Aluminum (Al)	Selenium (Se)
Iron (Fe)	Barium (Ba)	Lithium (Li)	Lithium (Li)
Arsenic (As)	Arsenic (As)	Arsenic (As)	Arsenic (As)
Molybdenum (Mo)	Molybdenum (Mo)	Iron (Fe)	Iron (Fe)
Chromium (Cr)	Cobalt (Co)	Cadmium (Cd)	Nickel (Ni)

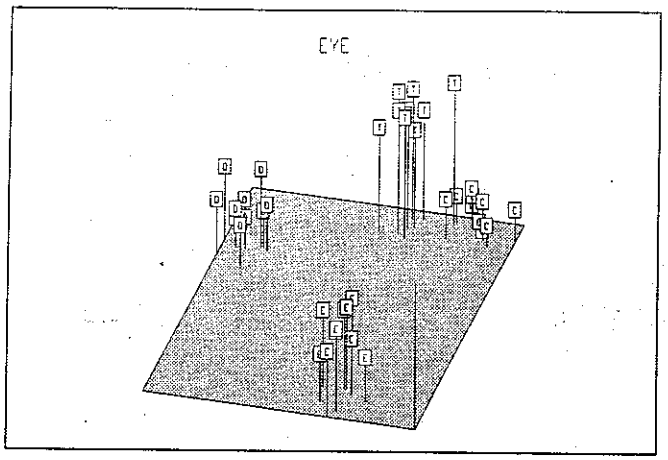

Fig. 3a.

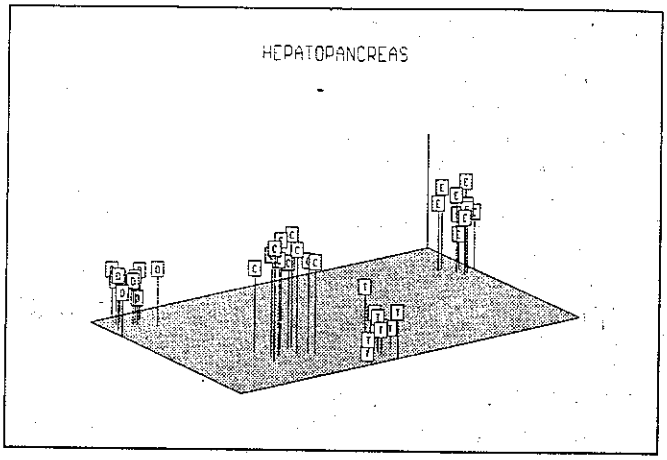

Fig. 3b.

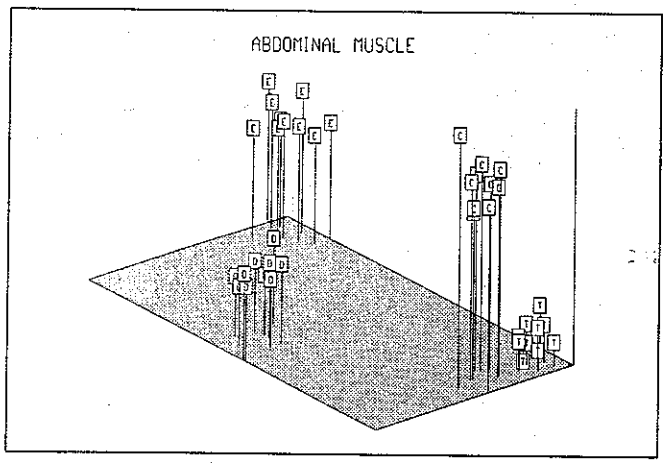

Fig. 3c.

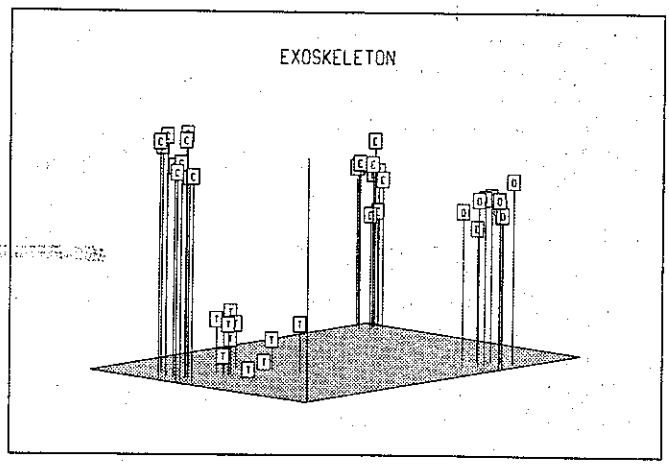

Fig. 3d.

Fig. 3a–d. *Canonical variate analyses based on the concentrations of 15 elements in juvenile eastern king prawns (Penaeus plebejus) from four nursery habitats. Four body tissue types were used (A, eye; B, Hepatopancreas; C, Abdominal Muscle; and D, Exoskeleton).*
The letters refer to the four nursery areas (E = Elliott River, D = Deception Bay, T = Tweed River and C = Clarence River).
The three unlabeled axes represent discriminant functions 1, 2, and 3.

whether the prawns sampled from each particular nursery area can be discriminated from prawns in other sites within the same area, say at different distances upstream. It would also be naive to conclude that the results provide information on the prawns' past environment. The analyses may simply reflect the elemental composition of the prawns at a certain place and at a certain time.

CONCLUSION

The study shows that ICP-MS can be used to measure the concentration of elements in various juvenile prawn body tissues and that combinations of these element concentrations can be used as discriminating variables to distinguish prawns from the different areas they currently inhabit. The value of this methodology for determining a geographic history of the prawns has yet to be demonstrated. Further experiments and analyses of these data are in progress to determine if the prawns retain elemental concentrations or profiles and to determine key elements that are likely to reflect geographic habitat.

ACKNOWLEDGMENT

We would like to thank New South Wales Fisheries and Fisheries Patrol Officers for permission to sample the prawns in the Clarence and Tweed Rivers, located in that state. Mr. Hugh Mawhinney and Mr. Paul Hielscher of the Queensland Department of Primary Industries (QDPI), Animal Research Institute, performed the ICP-MS analyses. Dr. Nigel Preston of the Commonwealth Scientific and Industrial Research Organisation (CSIRO), Division of Fisheries Research, provided suggestions about the design of the study. The study was funded by the Fisheries Research and Development Corporation (FRDC).

Received September 5, 1993.

REFERENCES

1. J.W. Penn, *Australian Journal of Marine and Freshwater Research* **26**, 197 (1975).
2. B.J. Hill and T.J. Wassenberg, *Fisheries Research* **3**, 223 (1985).
3. J.S. Edmonds, M.J. Moran, N. Caputi, and M. Morita, *Canadian Journal of Fisheries and Aquatic Sciences* **46**, 50 (1989).
4. J.S. Edmonds, N. Caputi, and M. Morita, *Australian Journal of Marine and Freshwater Research* **42**, 383 (1991).
5. J.N.C. Whyte and J.A. Boutillier, *Canadian Journal of Fisheries and Aquatic Sciences* **48**, 382 (1991).
6. N.V. Ruello, *Australian Journal of Marine and Freshwater Research* **26**, 343 (1975).
7. M.A. Potter, "Movements of the eastern king prawn (*Penaeus plebejus*) in southern Queensland waters," in *First Australian National Prawn Seminar* (Ed., P.C. Young), Australian Government Publishing Service, Canberra, pp. 10–17 (1975).
8. J.P. Glaister, T. Lau, and V.C. McDonall, *Australian Journal of Marine and Freshwater Research* **38**, 225 (1987).
9. S.S. Montgomery, *Fisheries Research* **9**, 189 (1990).
10. P. Greenway, *Biological Reviews* **60**, 425 (1985).
11. R.G. Galois, *Journal of Experimental Marine Biology and Ecology* **84**, 155 (1984).
12. S.L. Anderson, W.H. Clark, Jr., and E.S. Chang, *Biological Bulletin* **168**, 377 (1985).